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(54) Title: <b>CELL ACTIVATION PROCESS AND REAGENTS THEREFOR</b>			
(57) Abstract			
<p>A cell activation process is described in which an effector cell is transformed with DNA coding for a chimeric receptor containing two or more different cytoplasmic signalling components. The activated cell may be of use in medicine for example in the treatment of diseases such as cancer.</p>			

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CELL ACTIVATION PROCESS AND REAGENTS THEREFOR

This invention relates to a process for activating cells, a DNA delivery system for achieving cell activation and the use of activated cells in medicine.

- The natural T-cell receptor is a complex association of polypeptide chains comprising antigen binding, transmembrane and cytoplasmic components.
- 10 Binding of antigen to the receptor in the correct context triggers a series of intracellular events leading to activation of the T-cell and for example destruction of the antigen presenting target cell. Before recognition of the antigen can take place, the antigen must be presented in association with MHC molecules.
- 15 It would be highly desirable if this requirement for MHC could be bypassed by engineering T-cells to become active on binding ligands other than a natural MHC-presented antigen. This would provide a means of avoiding the variability between individuals associated with MHC presentation and
- 20 would also permit the targeting of more highly expressed surface antigens thereby increasing the efficacy of lymphocyte mediated therapy, for example in tumour therapy.
- 25 Chimeric receptors have been designed to target T-cells to cells expressing antigen on their cell surface. Such recombinant chimeric receptors include chimeras containing binding domains from antibodies and intracellular signalling domains from the T-cell receptor, termed 'T-bodies' [see for example Published International Patent Specifications Nos. WO 92/10591, WO 92/15322, WO 93/19163 and WO 95/02686].
- 30 The recombinant chimeric receptors described in the art are composed of a ligand binding component, a transmembrane component and a cytoplasmic component. It has been found however, that transfection of T-cells with these recombinant chimeric receptors does not result in acceptable levels of T-cell activation upon antigen binding unless the T-cell is also co-stimulated by, for example, treatment with high levels of

interleukin 2 [II-2]. The need for co-stimulation makes the method suitable principally for ex-vivo treatment of patients. This is a lengthy and complicated procedure.

- 5 The present invention offers an alternative to the present ex-vivo approach in that it achieves improved ex-vivo activation without the need for addition of costimulating agents such as II-2. It also advantageously provides successful in-vivo redirection and activation of T-cells, particularly in response to a single type of extracellular interaction.

10

Essentially the invention provides an effector cell which has been transformed with DNA coding for a chimeric receptor. The chimeric receptor contains two or more different signalling cytoplasmic components which are not naturally linked and which advantageously are chosen to act

- 15 together cooperatively to produce improved activation of the cell. DNA coding for such recombinant chimeric receptors may be introduced into T-cells or other effector cells in-vivo and/or ex-vivo. Subsequent binding of an effector cell expressing one or more of these chimeric receptors to a target cell elicits signal transduction leading to activation of the effector cell  
20 in a process involving clustering or dimerisation of chimeric receptors or allosteric changes in the chimeric receptor or another mechanism for receptor-triggering.

- 25 Thus according to one aspect of the invention we provide a method of activating a cell as a result of one type of extracellular interaction between said first cell and a molecule associated with a second target cell characterised in that said first cell is provided with a DNA delivery system comprising DNA coding for one or more recombinant chimeric receptors comprising two or more different cytoplasmic signalling components,  
30 wherein said cytoplasmic components are not naturally linked, and at least one is derived from a membrane spanning polypeptide.

- The DNA coding for the chimeric receptor(s) is arranged such that when it is expressed, and on the extracellular interaction between the cell and a  
35 second target cell, a signal is transduced via the cytoplasmic signalling components to two or more different intracellular signalling messengers.

This results in activation of the cell and elicits a biological response to the target cell. As used herein, cell activation means activation of one or more signal transduction pathways. This may be evidenced by an increase in cell proliferation; expression of cytokines with, for example pro or anti-inflammatory responses; stimulation of cytolytic activity, differentiation or other effector functions; antibody secretion; phagocytosis; tumour infiltration and/or increased adhesion.

5       The cytoplasmic signalling components are preferably selected such that they are capable of acting together cooperatively. They are "not naturally linked", which term is used herein to denote cytoplasmic signalling components which in nature are not connected to each other on a single polypeptide chain. Particularly useful signalling components include those described hereinafter in relation to other aspects of the invention.

10      15     In addition to the cytoplasmic signalling components each recombinant chimeric receptor preferably comprises a binding component capable of recognising a cell surface molecule on a target cell, and a transmembrane component. The DNA coding for these components will additionally code for a signal peptide to ensure that the chimeric receptor(s) once expressed will be directed to the cell surface membrane. All the components may be coded for by a single DNA coding sequence.

20      25     Alternatively, each cytoplasmic signalling component may be coded for by two or more separate DNA coding sequences. In this instance each DNA coding sequence may also code for a signal peptide, a transmembrane component and/or a binding component. The binding components may be different, but will generally all be capable of participating in the same type of extracellular event, for example by binding to the same molecule associated with the target cell. In one preference the binding components are the same.

30      35     In some of the applications described hereinafter, for example where the binding component is an antibody or an antibody fragment, the DNA coding for the chimeric receptor may comprise two separate DNA coding sequences, one sequence for example coding for part of the binding

component [in the case of an antibody for example a  $V_H$  component] linked to the signal peptide, transmembrane and cytoplasmic signalling component(s), and the second sequence coding for the remainder of the binding component [for example a  $V_L$  component in the example given].

5

In order to activate a desired cell the DNA coding for the chimeric receptor will first need to be delivered to the cell. Thus according to a second aspect of the invention we provide a DNA delivery system comprising DNA in association with a carrier said DNA coding for a recombinant

10 chimeric receptor capable of one type of extracellular interaction and comprising two or more different cytoplasmic signalling components which are not naturally linked, and wherein at least one of said cytoplasmic components is derived from a membrane spanning polypeptide.

15 In this aspect of the invention the chimeric receptor may be coded for by a single DNA coding sequence, coding in particular for the two or more different cytoplasmic signalling components. Thus in one preference the invention provides a DNA delivery system comprising DNA in association with a carrier said DNA coding for a recombinant chimeric receptor  
20 wherein said DNA codes in reading frame for:

- i) a signal peptide component;
- ii) a binding component capable of recognising a cell surface molecule on a target cell;

25 iii) a transmembrane component;  
iv) two or more different cytoplasmic signalling components which are not naturally linked, and wherein at least one of said cytoplasmic components is derived from a membrane spanning polypeptide, and optionally

30 v) one or more spacer regions linking any two or more of said i) to iv) components.

The components of the recombinant chimeric receptor are operatively linked such that the signalling cytoplasmic components are functional in

35 transducing a signal resulting in activation of one or more messenger

systems as a result of recognition of a cell surface molecule on a target cell by the binding component.

Two or more of the components may be linked by one or more spacer regions. The spacer regions may function to facilitate the components adopting the correct conformation for biological activity. The use of a spacer region to link the transmembrane component iii) and the binding component ii) is particularly advantageous.

10 The spacer regions may for example comprise up to 300 amino acids and preferably 20 to 100 amino acids and most preferably 25 to 50 amino acids.

15 Spacers may be derived from all or part of naturally occurring molecules such as from all or part of the extracellular region of CD8, CD4 or CD28; or all or part of an antibody constant region, including the hinge region. All or part of natural spacing components between functional parts of intracellular signalling molecules for example spacers between ITAMS (immunoreceptor tyrosine based activation motifs) may also be used.

20 Alternatively the spacer may be a non-naturally occurring sequence.

The binding component ii) may be any molecule capable of interacting with cell surface molecules and may be chosen to recognise a surface marker expressed on cells associated with a disease state such as for 25 example those associated with virally infected cells; bacterially infected cells; cancer cells, such as the bombesin receptor expressed on lung tumour cells, carcinoembryonic antigen, polymorphic epithelial mucin, and CD33; peptide hormones, adhesion molecules, inflammatory cells present in autoimmune disease, or a T-cell receptor or antigen giving rise to 30 autoimmunity.

Suitable binding components for use in the chimeric receptors of the invention also include all or part of receptors associated with binding to cell surface associated molecules; the T-cell receptor; CD4; CD8; CD28; 35 cytokine receptors e.g. an interleukin receptor, TNF receptor, or interferon receptor e.g.  $\gamma$ -IFN; receptors for colony stimulating factors e.g. GMCSF;

antibodies and antigen binding fragments thereof including for example Fab, Fab', F(ab')<sub>2</sub>, single chain Fv, Fv, and  $V_H$  or  $V_L$  components which may be in association with  $C_H$  and  $C_L$  domains. The antibodies or fragments may be murine, human, chimeric or engineered human  
5 antibodies and fragments. As used herein the term engineered human antibody or fragment is intended to mean an antibody or fragment which has one or more CDR's and one or more framework residues derived from one antibody, e.g. a murine antibody embedded in an otherwise human framework. Such antibodies are well known and may be prepared by a  
10 number of methods for example as described in International Patent Specification No. WO91/09967.

Particularly useful binding components include Fab' fragments or, especially, single chain Fv fragments.

15

When the binding component is an antibody or antibody fragment other than a single chain Fv or  $V_H$  or  $V_L$  component which contains separate binding chains it will be necessary to include a second separate DNA coding sequence in the delivery system according to the invention to code  
20 for the second binding chain. In this instance the first DNA sequence containing the cytoplasmic signalling components and one chain of the antibody or fragment will be coexpressed with the second DNA sequence coding for a signal peptide and the second chain of the antibody or fragment so that assembly of the antibody binding component can occur.

25

Transmembrane components iii) may be derived from a wide variety of sources such as all or part of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD8, CD4, a cytokine receptor, e.g. an interleukin receptor, TNF receptor, or interferon receptor, or a colony stimulating factor receptor e.g. GMCSF.

The binding and transmembrane components may be linked directly or, preferably, by a spacer region. The spacer region may be one or more of the regions described above. Where more than one region is present, for  
35 example two regions, these are preferably different regions, for example

an antibody hinge region linked to all or part of the extracellular region of CD28.

The spacer and transmembrane components are advantageously chosen  
5 such that they have free thiol groups thereby providing the chimeric receptor with multimerisation, particularly dimerisation capacity. Receptors of this type, especially dimers, are particularly preferred and include those which have CD28 components, the zeta chain of the natural T-cell receptor, and/or antibody hinge sequences.

10

The transmembrane component may or may not be naturally linked to the cytoplasmic component to which it is attached either directly or by means of a spacer.

15

The cytoplasmic signalling components iv) can for example transduce a signal which results in activation of one or more intracellular messenger systems. It is preferred that each of the cytoplasmic components activates a different messenger system. The intracellular messenger systems which may be activated either directly or indirectly include, for example,  
20 one or more kinase pathways such as those involving tyrosine kinase, PKC or MAP kinase; G-protein or phospholipase mediated pathways; calcium mediated pathways; and pathways involving synthesis of a cytokine such as an interleukin e.g. IL-2, including NFAT, and cAMP mediated pathways.

25

Examples of suitable cytoplasmic components iv) include, for example those derived from the T-cell receptor such as all or part of the zeta, eta or epsilon chain; CD28; the  $\gamma$  chain of a Fc receptor; or signalling components from a cytokine receptor e.g. interleukin, TNF and interferon  
30 receptors, a colony stimulating factor receptor e.g. GMCSF, a tyrosine kinase e.g. ZAP-70, fyn, lyk, Itk and syk; an adhesion molecule e.g. LFA-1 and LFA-2, B29, MB-1, CD3 delta, CD3 gamma, CD5 or CD2. The signalling cytoplasmic components are preferably ITAM containing cytoplasmic components

35

The cytoplasmic signalling components are preferably selected so that they act cooperatively. They may be in any orientation relative to one another. Particularly useful components include all or part of the signalling component of CD28 or the zeta chain of the T-cell receptor.

5

The signal component may be that naturally associated with the binding component or may be derived from other sources.

Examples of suitable signal peptide components i) include immunoglobulin  
10 signal sequences.

The signal component, binding component, transmembrane component, and cytoplasmic components are preferably derived from or based on human sequences.

15

Homologues of the individual components of the chimeric receptor may be used and the invention is to be understood to extend to such use. The term homologue as used herein with respect to a particular nucleotide or amino acid sequence coding for a component of the chimeric receptor

20 represents a corresponding sequence in which one or more nucleotides or amino acids have been added, deleted, substituted or otherwise chemically modified provided always that the homologue retains substantially the same function as the particular component of the chimeric receptor. Homologues may be obtained by standard molecular  
25 biology and/or chemistry techniques e.g. by cDNA or gene cloning, or by use of oligonucleotide directed mutagenesis or oligonucleotide directed synthesis techniques or enzymatic cleavage or enzymatic filling in of gapped oligonucleotides.

30 Fragments of the individual components may also be used wherein one or more nucleotides has been deleted provided that the fragment retains substantially the same function as the starting component of the chimeric receptor.

35 The DNA for use in this and other aspects of the invention may be obtained from readily available DNA sources using standard molecular

biology and/or chemistry procedures, for example by use of oligonucleotide directed mutagenesis or oligonucleotide directed synthesis techniques, enzymatic cleavage or enzymatic filling in of gapped oligonucleotides. Such techniques are described by Maniatis *et al* in

5 Molecular Cloning, Cold Spring Harbor Laboratory, New York 1989, and in particular in the Examples hereinafter.

The carrier for use in the DNA delivery systems according to the invention may be a vector or other carrier suitable for introduction of the DNA *ex-vivo* or *in-vivo* into target cells and/or target host cells. Examples of

10 suitable vectors include viral vectors such as retroviruses, adenoviruses, adenoassociated viruses, EBV, and HSV, and non-viral vectors, such as liposomal vectors and vectors based on DNA condensing agents. Alternatively the carrier may be an antibody. Where appropriate, the

15 vector may additionally include promoter/regulatory sequences and/or replication functions from viruses such as retrovirus LTRs, AAV repeats, SV40 and hCMV promoters and/or enhancers, splicing and polyadenylation signals; EBV and BK virus replication functions. Tissue specific regulatory sequences such as the TCR- $\alpha$  promoter, E-selectin

20 promoter and the CD2 promoter and locus control region may also be used.

Where two or more DNA molecules are used in the DNA delivery system they may be incorporated into the same or different carriers as described

25 above.

For *ex-vivo* use, the DNA delivery system of the invention may be introduced into effector cells removed from the target host using methods well known in the art e.g. transfection, transduction, biolistics, protoplast

30 fusion, calcium phosphate precipitated DNA transformation, electroporation, cationic lipofection, or targeted liposomes. The effector cells are then reintroduced into the host using standard techniques.

A wide variety of target hosts may be employed according to the present

35 invention such as, for example, mammals and, especially, humans.

Examples of suitable effector cells include cells associated with the immune system such as lymphocytes e.g. cytotoxic T-lymphocytes, tumour infiltrating lymphocytes, natural killer cells, neutrophils, basophils or T-helper cells; dendritic cells, B-cells, haemoatopoietic stem cells, 5 macrophages, monocytes or NK cells. The use of cytotoxic T-lymphocytes is especially preferred.

The DNA delivery system according to the invention is particularly suitable for *in vivo* administration. It may be in one preferred example in the form 10 of a targeted delivery system in which the carrier is capable of directing the DNA to a desired effector cell. Particular examples of such targeted delivery systems include targeted-naked DNA, targeted liposomes encapsulating and/or complexed with the DNA, targeted retroviral systems and targeted condensed DNA such as protamine and polylysine 15 condensed DNA.

Targeting systems are well known in the art and include using, for example, antibodies or fragments thereof against cell surface antigens expressed on target cells *in vivo* such as CD8; CD16; CD4; CD3; selectins 20 e.g. E-selectin; CD5; CD7; CD34; activation antigens e.g. CD69 and IL-2R. Alternatively, other receptor - ligand interactions can be used for targeting e.g. CD4 to target HIV<sub>gp</sub>160 - expressing target cells.

In general the use of antibody targeted DNA is preferred, particularly 25 antibody targeted naked DNA, antibody targeted condensed DNA and especially antibody targeted liposomes. Particular types of liposomes which may be used include for example pH-sensitive liposomes where linkers cleaved at low pH may be used to link the antibody to the liposome. Cationic liposomes which fuse with the cell membrane and deliver the 30 recombinant chimeric receptor DNA according to the invention directly into the cytoplasm may also be used. Liposomes for use in the invention may also have hydrophilic groups attached to their surface to increase their circulating half-life such as for example polyethylene glycol polymers. There are many examples in the art of suitable groups for attaching to 35 liposomes or other carriers; see for example International Patent

91/05546, WO 93/19738, WO 94/20073 and WO 94/22429. The antibody or other targeting molecule may be linked to the DNA, condensed DNA or liposome using conventional readily available linking groups and reactive functional groups in the antibody e.g. thiols, or amines and the like, and in  
5 the DNA or DNA containing materials.

Non-targeted delivery systems may also be used and in these targeted expression of the DNA is advantageous. Targeted expression of the DNA may be achieved for example by using T-cell specific promoter systems  
10 such as the zeta promoter and CD2 promoter and locus control region, and the perforin promoter.

The aspect of the invention described above advantageously utilises a single DNA sequence to code for the chimeric receptor. It will be  
15 appreciated however that the invention may be extended to DNA delivery systems in which the chimeric receptor is coded for by two or more separate DNA coding sequences. Thus in one example, a first and second separate DNA coding sequence may be present in the delivery system each of which codes for components i) to iv) and optionally v) in  
20 the same reading frame as described above but which differ from each other in that the cytoplasmic signalling component iv) is not the same. The two DNA coding sequences may each code for more than one signalling component providing that at least one component on the first DNA is different to any other signalling component on the second DNA.  
25 As above, the signalling components are advantageously selected to act cooperatively and the remaining components may be any of those previously described for the single DNA embodiment. The binding component iv) coded for by the first DNA will preferably be the same as that coded for by the second DNA. Advantageously the binding  
30 component coded by the first DNA will be separated from the transmembrane component by a different spacer region to that coded by the second DNA.

35 The delivery system may be used ex vivo and in a further aspect the invention provides effector cells transfected with a DNA delivery system according to the invention. The effector cells may be any of those

previously described above which are suitable for ex vivo use and are preferably T-cells most preferably cytotoxic T-cells.

- 5      The DNA delivery system may take the form of a pharmaceutical composition. It may be a therapeutic or diagnostic composition and may take any suitable form suitable for administration. Preferably it will be in a form suitable for parenteral administration e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the composition is for injection or infusion, it may take the form of a suspension, solution or  
10     emulsion in an oily or aqueous vehicle and it may contain formulation agents such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the composition may be in dry form, for reconstitution before use with an appropriate sterile liquid.
- 15     If the composition is suitable for oral administration the formulation may contain, in addition to the active ingredient, additives such as: starch - e.g. potato, maize or wheat starch or cellulose - or starch derivatives such as microcrystalline cellulose; silica; various sugars such as lactose; magnesium carbonate and/or calcium phosphate. It is desirable that, if the  
20     formulation is for oral administration it will be well tolerated by the patient's digestive system. To this end, it may be desirable to include in the formulation mucus formers and resins. It may also be desirable to improve tolerance by formulating the compositions in a capsule which is insoluble in the gastric juices. It may also be preferable to include the  
25     composition in a controlled release formulation.

30     The DNA delivery system according to the invention is of use in medicine and the invention extends to a method of treatment of a human or animal subject, the method comprising administering to the subject an effective amount of a DNA delivery system described above. The exact amount to be used will depend on the ages and condition of the patient, the nature of the disease or disorder and the route of administration, but may be determined using conventional means, for example by extrapolation of animal experiment derived data. In particular, for ex vivo use the number  
35     of transfected effector cells required may be established by ex vivo transfection and re-introduction into an animal model of a range of effector

cell numbers. Similarly the quantity of DNA required for *in vivo* use may be established in animals using a range of DNA concentrations.

- The DNA delivery system according to the invention may be useful in the treatment of a number of diseases or disorders. Such diseases or disorders may include those described under the general headings of infectious diseases, e.g. HIV infection; inflammatory disease/autoimmunity e.g. rheumatoid arthritis, osteoarthritis, inflammatory bowel disease; cancer; allergic/atopic diseases e.g. asthma, eczema; congenital e.g. 5 cystic fibrosis, sickle cell anaemia; dermatologic, e.g. psoriasis; neurologic, e.g. multiple sclerosis; transplants e.g. organ transplant rejection, graft-versus-host disease; metabolic/idiopathic disease e.g. diabetes.
- 10 15 DNA coding for a chimeric receptor as described herein also forms a feature of the invention, particularly for use in a delivery system described herein.

The invention is further illustrated in the following non-limiting Examples 20 and Figures in which:

- Figure 1 shows: diagrammatic representation of recombinant chimeric receptor constructs cloned into pBluescript SK+
- 25 Figure 2 shows: diagrammatic representation of recombinant chimeric receptor constructs cloned into pBluescript SK+
- Figure 3 shows: oligonucleotide sequences for recombinant chimeric receptor construction
- Figure 4 shows: nucleotide and amino acid sequence of an hCTMO1/CD8/zeta recombinant chimeric receptor
- 30 Figure 5 shows: nucleotide and amino acid sequence of an hCTMO1/CD8/zeta-CD28 recombinant chimeric receptor fusion
- Figure 6 shows: nucleotide and amino acid sequence of an hCTMO1/CD8/CD28 recombinant chimeric receptor
- 35 Figure 7 shows: nucleotide and amino acid sequence of an CTMO1/G1/zeta recombinant chimeric receptor

Figure 8 shows: nucleotide and amino acid sequence of an hCTMO1/G1/zeta-CD28 recombinant chimeric receptor fusion

Figure 9 shows: nucleotide and amino acid sequence of an hCTMO1/h/CD28 recombinant chimeric receptor

5 Figure 10 shows: histogram representation of IL2 production by cell lines TB3.2, 3.13 and 3.24 when stimulated with an anti-idiotypic antibody alone or in combination with an anti-CD28 antibody

10 Figure 11 shows: histogram representation of the production of IL2 by cell line TB3.13 when stimulated with antigen expressing tumour cells, shown with and without co-stimulation using an anti-CD28 antibody.

Figure 12 shows: histogram representation of IL-2 production by HGT1.2 and HGT1.4 in response to various stimuli

15 Figure 13 shows: histogram representation of IL-2 production by HGT2.4 incubated with various combinations of antibodies.

Figure 14 shows: schematic representation of recombinant chimeric receptor constructs.

20 Figure 15 shows: schematic representation of recombinant chimeric receptor constructs

Figure 16 shows: schematic representation of recombinant chimeric receptor constructs.

25 Figure 17 shows: schematic representation of recombinant chimeric receptor constructs

Figure 18 shows: histogram representation of levels of expression of CD28 chimeras in Jurkat cells

30 Figure 19 shows: histogram representation of IL-2 production by Jurkat cells expressing two different chimeric receptors in response to target cells.

Figure 20 shows: Graph showing Cytolysis of target cells by CD8+ve human CTL cells infected with recombinant adenoviruses

5    **EXAMPLE 1**

**Construction of chimeric receptor genes**

Each component of the chimeric receptor constructs was either PCR cloned or PCR assembled by standard techniques (PCR Protocols, Innis et al, 1990, Academic Press inc. ) and sub-cloned in a cassette format into 10 pBluescript SK+ (Stratagene), see figure 1, 2, 2b and 2c. Oligonucleotides are described in Figure 3.

1.    **Single chain Fv cassettes**

**hCTMO1**

15    An scFv from the engineered human CTMO1 antibody was constructed as follows. Leader sequence and hCTMO1 VI was PCR cloned from plasmid pAL 47 (International Patent Specification No. WO 93/06231) with oligos R6490 and R6516 (Oligo sequences are shown in Figure 3). R6490 introduces 5' Not I and Hind III sites and R6516 forms part of the 20 (Gly4Ser)5 linker. hCTMO1 Vh was PCR cloned from plasmid pAL 52 (WO 93/06231) with oligos R6515 (forms part of linker) and R6514 (introduces 3' Spe I site. Leader / VI and Vh fragments were then PCR spliced together and the PCR product was restricted with Not I and Spe I and sub-cloned into pBluescript SK+.

25

**hP67.6**

An scFv from another engineered human antibody, hP67.6, engineered according to WO91/09967, was similarly prepared and subcloned into pBluescript SK+.

30

2.    **CD8 hinge spacer cassette**

The CD8 hinge spacer for hCTMO1 TCR Zeta chimeric receptor and hCTMO1 TCR Zeta-CD28 fusion chimeric receptor (which includes a small part of 5' Zeta) was PCR assembled using overlapping oligos: 35 R6494,R6495,R6496 and R6497. The CD8 hinge spacer for hCTMO1 CD28 chimeric receptor was PCR assembled using overlapping oligos:

R6494, R6495, R6496 and R6506. Both PCR products were restricted with Spe I and BamH I and sub-cloned into pBluescript SK+.

3. Human TCR Zeta cassette

5 Human Zeta transmembrane and intracellular components were PCR cloned from human leukocyte cDNA (Clonetech) with oligos R6488 (introducing a 5' BamH I site) and R6489 (introducing a 3' EcoR I site). PCR product was restricted with BamH I and EcoR I and sub-cloned into pBluescript SK+.

10

4. Human CD28 cassette

Human CD28 transmembrane and intracellular components were PCR cloned from human leukocyte cDNA (Clonetech) with oligos P3240 (introducing a 5' BamH I site) and P3241 (introducing a 3' EcoR I site).

15 PCR product was restricted with BamH I and EcoR I and sub-cloned into pBluescript SK+.

5. Hinge-CD28 cassette

Human CD28 extracellular, transmembrane and intracellular components 20 were PCR cloned from human leukocyte cDNA (Clonetech) with oligos S0146 (introducing a 5' Spe I site) and P3241 (introducing a 3' EcoR I site). S0146 also constitutes residues 234 to 243 of human IgG1 hinge. The product of the PCR reaction was digested with restriction enzyme SpeI and EcoR1 and sub-cloned into pBluescriptSK+.

25

6. Zeta-CD28 fusion cassette

The 3' end of Zeta, starting at a naturally occurring Sty I site and the intracellular component of human CD28 were PCR assembled such that the Zeta stop codon was removed and an inframe fusion protein would be 30 translated. PCR assembly carried out with overlapping oligos: P3301, P3302, P3303, P3304, P3305 and P3306. PCR product was restricted with Sty I and EcoR I and sub-cloned into pBluescriptSK+ containing the hCTMO1 TCR Zeta chimeric receptor construct, replacing the 3' end of Zeta.

35

7. Human IgG1 spacer cassette

Human IgG1 hinge, CH2 and CH3 were PCR cloned from IgG1 cDNA clone (A. Popplewell) with oligos S0060 (introducing a 5' Spe I site) and S0061 (introducing residues L, D, P, and K constituting a 3' BamH I site). PCR product was restricted with Spe I and BamH I and sub-cloned into 5 pBluescriptSK+.

8. h.28 spacer cassette

- Human IgG1 hinge and part of human CD28 extracellular component were PCR cloned from a scFv/h/CD28 plasmid with oligos T4057 and T4058. 10 T4057 introduces a 5' Spe I site and T4058 introduces residues L, D, P, and K constituting a 3' BamH I site. PCR product was restricted with Spe I and BamH I and sub-cloned into pBluescriptSK+.

9. CD28-Zeta fusion cassette

- 15 Human CD28 transmembrane and intracellular components were PCR cloned from a scFv/h/CD28 plasmid with oligos T7145 and T4060. T7145 introduces residues L, D, P, and K constituting a 3' BamH I site. T4060 comprises a 3' overhang compatible with the 5' end of human Zeta intracellular component.
- 20 Human Zeta intracellular component was PCR cloned from a scFv/G1/Zeta plasmid with oligos T4387 and S4700. T4387 comprises a 5' overhang compatible with the 3' end of human CD28 intracellular component. S4700 introduces a 3' EcoR I site.
- 25 CD28 transmembrane and intracellular components were then PCR spliced to Zeta intracellular component with oligos T7145 and S4700. PCR product was restricted with BamH I and EcoR I and sub-cloned into pBluescriptSK+.

10. CD28-Zeta-CD28 fusion cassette

- 30 A Pst I restriction site in human Zeta was used to subclone the 3' end of Zeta intracellular component and the CD28 intracellular component on a Pst I to EcoR I fragment ifrom the Zeta-CD28 fusion cassette into the CD28-Zeta fusion cassette, replacing the 3' end of Zeta. This generates a CD28-Zeta-CD28 fusion cassette with a 5' BamH I site and 3' EcoR I site.

All of the above cassettes were completely sequenced (Applied Biosystems, Taq DyeDeoxy Terminator Cycle Sequencing, Part Number 901497) in pBluescriptSK+ prior to cloning into the expression vectors.

- 5 These cassettes were assembled to construct chimeric receptors with the specificity of the engineered human antibodies hCTMO1, directed against human polymorphic epithelial mucin (PEM) or hP67.6, directed against human CD33, by assembling the appropriate cassettes using standard molecular biology techniques. The following chimeric receptors were  
10 constructed; see Table 2 and Figures 14 - 17 in which potential di-sulphide bonds are indicated by a horizontal line between the two sub-units (not all di-sulphide bonds may form in 100% of the molecules).
- 15 1) **scFv / CD8 / Zeta Chimeric Receptor (Figure 14)**  
The scFv / CD8 / Zeta chimeric receptor consists of a single chain Fv (scFv) linked to an extracellular spacer in the form of part of human CD8 hinge, linked to the extracellular, transmembrane and intracellular components of the human T-cell receptor Zeta chain (TCR).
- 20 20 The scFv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a (Gly4Ser)5 linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues 98 to 142 of the hinge region of human CD8 (Zamoyska et al : Cell 43, 153-163, 1985  
25 ). This is linked to residues 6 to 142 of human TCR Zeta comprising extracellular (part), transmembrane and intracellular regions (Weissman et al : PNAS 85, 9709-9713, 1988. Moingeon et al: Eur. J. Immunol. 20, 1741-1745, 1990).
- 30 2) **scFv / CD8 / CD28 Chimeric Receptor (Figure 14)**  
The CD8 hinge/CD28 chimeric receptor consists of a scFv linked to an extracellular spacer in the form of part of human CD8 hinge, linked to the transmembrane and intracellular component of human CD28.
- 35 35 The scFv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a (Gly4Ser)5

linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues 98 to 142 of the hinge region of human CD8 (Zamoyska *et al* : Cell 43 153-163, 1985 ). This is linked to residues 132 to 202 of human CD28 comprising  
5 the transmembrane and intracellular components (Aruffo & Seed : PNAS 84, 8573-8577).

3) **scFv /CD8 / Zeta-CD28 Fusion Chimeric Receptor (Figure 14)**

The scFv /CD8 / Zeta-CD28 Fusion chimeric receptor consists of a single  
10 chain Fv linked to an extracellular spacer in the form of part of human CD8 hinge, linked to the extracellular, transmembrane and intracellular components of human TCR Zeta fused to the intracellular component of human CD28.  
  
15 The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a (Gly4Ser)5 linker to the variable component of the heavy chain of the engineered human antibody. The extra cellular spacer consists of residues 98 to 142 of the hinge region of human CD8 (Zamoyska *et al* : Cell,  
20 43,153-163, 1985). This is linked to residues 6 to 142 of human TCR Zeta comprising extracellular (part), transmembrane and intracellular components (Weissman *et al* : PNAS 85,9709-9713, 1988 Moingeon *et al*:Eur. J. Immunol. 20, 1741-1745, 1990).  
This is linked to residues 162 to 202 comprising the intracellular  
25 component of human CD28.

4) **scFv / G1 / Zeta Chimeric Receptor (Figure 15)**

The scFv / G1 / Zeta chimeric receptor consists of a single chain Fv linked to an extracellular spacer comprising human IgG 1 hinge, CH2 and CH3,  
30 linked to the transmembrane and intracellular regions of human TCR Zeta.

The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a (Gly4Ser)5 linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues 234 to 243 of human IgG1 hinge, 244 to 360 of CH2 and 361 to 478 of

CH3 (Kabat *et al* Sequences of proteins of immunological interest, 1987 ). This is linked to residues 6 to 142 of human TCR Zeta comprising extracellular (part), transmembrane and intracellular regions (Weissman *et al* : PNAS 85, 9709-9713, 1988. Moingeon *et al*: Eur. J. Immunol. 20, 1741-5 1745, 1990).

5) **scFv / G1 / Zeta-CD28 fusion Chimeric Receptor (Figure 15)**  
The scFv / G1 / Zeta chimeric receptor consists of a single chain Fv linked to an extracellular spacer comprising human IgG 1 hinge, CH2 and CH3, 10 linked to the transmembrane and intracellular regions of human Zeta fused to the intracellular region of human CD28.

The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via 15 a (Gly4Ser)5 linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues 234 to 243 of human IgG1 hinge, 244 to 360 of CH2 and 361 to 478 of CH3 (Kabat *et al* Sequences of proteins of immunological interest, 1987 ). This is linked to residues 6 to 142 of human TCR Zeta comprising 20 extracellular (part), transmembrane and intracellular regions (Weissman *et al* : PNAS 85, 9709-9713, 1988. Moingeon *et al*: Eur. J. Immunol. 20, 1741-1745, 1990).  
This is linked to residues 162 to 202 comprising the intracellular component of human CD28 (Aruffo & Seed : PNAS 84, 8573-8577).

25  
6) **scFv / h / CD28 Chimeric Receptor (Figure 15)**  
The scFv / h / CD28 chimeric receptor consists of a single chain Fv linked to an extracellular spacer consisting of human IgG1 hinge and part of the extracellular region of human CD28, linked to the transmembrane and 30 intracellular regions of human CD28.

The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a (Gly4Ser)5 linker to the variable component of the heavy chain of the 35 engineered human antibody. The extracellular spacer consists of residues 234 to 243 of human IgG1 hinge and residues 118 to 134 of human CD28.

This is linked to residues 135 to 202 of human CD28 comprising the transmembrane and intracellular regions (Aruffo & Seed : PNAS 84, 8573-8577).

5 7) scFv / G1 / CD28 Chimeric Receptor (Figure 16)

The scFv / G1 / Zeta chimeric receptor consists of a single chain Fv linked to an extra cellular spacer comprising human IgG 1 hinge, CH2 and CH3, linked to the transmembrane and intracellular regions of human CD28.

10 The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a (Gly4Ser)5 linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues 234 to 243 of human IgG1 hinge, 244 to 360 of CH2 and 361 to 478 of  
15 CH3 (Kabat *et al* Sequences of proteins of immunological interest, 1987 ). This is linked via residues L, D, P and K to residues 135 to 202 comprising the transmembrane and intracellular components of human CD28 (Aruffo & Seed : PNAS 84, 8573-8577).

20 8) scFv / G1 / CD28 -Zeta fusion Chimeric Receptor (Figure 16)

The scFv / G1 / Zeta chimeric receptor consists of a single chain Fv linked to an extracellular spacer comprising human IgG 1 hinge, CH2 and CH3, linked to the transmembrane and intracellular regions of human CD28 fused to the intracellular region of human Zeta.

25 The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a (Gly4Ser)5 linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues  
30 234 to 243 of human IgG1 hinge, 244 to 360 of CH2 and 361 to 478 of CH3 (Kabat *et al* Sequences of proteins of immunological interest, 1987 ). This is linked via residues L, D, P and K to residues 135 to 202 comprising the transmembrane and intracellular components of human CD28.  
This is linked to residues 31 to 142 of human TCR Zeta, the intracellular  
35 region (Weissman *et al* : PNAS 85 ,9709-9713, 1988. Moingeon *et al*:Eur. J. Immunol. 20, 1741-1745, 1990).

9) **scFv / G1 / CD28 -Zeta -CD28 fusion Chimeric Receptor (Figure 16)**

The scFv / G1 / Zeta chimeric receptor consists of a single chain Fv linked  
5 to an extracellular spacer comprising human IgG 1 hinge, CH2 and CH3,  
linked to the transmembrane and intracellular regions of human CD28  
fused to the intracellular region of human Zeta fused to the intracellular  
region of CD28.

- 10 The single chain Fv consists of the leader sequence and variable  
component of the light chain of the engineered human antibody linked via  
a (Gly4Ser)5 linker to the variable component of the heavy chain of the  
engineered human antibody. The extracellular spacer consists of residues  
234 to 243 of human IgG1 hinge, 244 to 360 of CH2 and 361 to 478 of  
15 CH3 (Kabat *et al* Sequences of proteins of immunological interest, 1987 ).  
This is linked via residues L, D, P and K to residues 135 to 202 comprising  
the transmembrane and intracellular components of human CD28.  
This is linked to residues 31 to 142 of human TCR Zeta, the intracellular  
region (Weissman *et al* : PNAS 85 ,9709-9713, 1988. Moingeon *et al*:Eur.  
20 J. Immunol. 20, 1741-1745, 1990).  
This is linked to residues 162 to 202 comprising the intracellular  
component of human CD28.

10) **scFv / h.28 / Zeta Chimeric Receptor (Figure 17)**

- 25 The scFv / h / CD28 chimeric receptor consists of a single chain Fv linked  
to an extracellular spacer consisting of human IgG1 hinge, part of the  
extracellular region of human CD28 and 4 amino acid residues, linked to  
the transmembrane and intracellular regions of human TCR Zeta.
- 30 The single chain Fv consists of the leader sequence and variable  
component of the light chain of the engineered human antibody linked via  
a (Gly4Ser)5 linker to the variable component of the heavy chain of the  
engineered human antibody. The extracellular spacer consists of residues  
234 to 243 of human IgG1 hinge and residues 118 to 134 of human CD28.
- 35 This is linked via residues L, D, P and K to residues 10 to 142 of human  
TCR Zeta comprising the transmembrane and the intracellular region

(Weissman *et al* : PNAS 85, 9709-9713, 1988. Moingeon *et al*: Eur. J. Immunol. 20, 1741-1745, 1990).

**11) scFv / h.28 / Zeta-CD28 fusion Chimeric Receptor (Figure 17)**

- 5 The scFv / h / CD28 chimeric receptor consists of a single chain Fv linked to an extracellular spacer consisting of human IgG1 hinge, part of the extracellular region of human CD28 and 4 amino acid residues, linked to the transmembrane and intracellular regions of human Zeta fused to the intracellular region of human CD28.

10

The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a (Gly4Ser)5 linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues

- 15 234 to 243 of human IgG1 hinge and residues 118 to 134 of human CD28. This is linked via residues L, D, P and K to residues 10 to 142 of human TCR Zeta comprising transmembrane and intracellular regions (Weissman *et al* : PNAS 85, 9709-9713, 1988. Moingeon *et al*: Eur. J. Immunol. 20, 1741-1745, 1990).

- 20 This is linked to residues 162 to 202 comprising the intracellular component of human CD28.

**12) scFv / h.28 / CD28-Zeta fusion Chimeric Receptor (Figure 17)**

- 25 The scFv / h / CD28 chimeric receptor consists of a single chain Fv linked to an extracellular spacer consisting of human IgG1 hinge, part of the extracellular region of human CD28 and 4 amino acid residues, linked to the transmembrane and intracellular regions of human CD28 fused to the intracellular region of human Zeta.

- 30 The single chain Fv consists of the leader sequence and variable component of the light chain of the engineered human antibody linked via a (Gly4Ser)5 linker to the variable component of the heavy chain of the engineered human antibody. The extracellular spacer consists of residues 234 to 243 of human IgG1 hinge and residues 118 to 134 of human CD28.

- 35 This is linked via residues L, D, P and K to residues 135 to 202 comprising the transmembrane and intracellular components of human CD28.

This is linked to residues 31 to 142 of human TCR Zeta, the intracellular region (Weissman *et al* : PNAS 85, 9709-9713, 1988. Moingeon *et al*: Eur. J. Immunol. 20, 1741-1745, 1990).

- 5 Table 1 shows a number of preferred recombinant chimeric receptors which may be made in an analogous way by following the above teaching and methods.

- Table 2 gives details of the chimeric receptor constructs and cell line  
10 nomenclature used.

#### EXAMPLE 2

##### Analysis of hCTMO1-chimeric receptor constructs expressed in Jurkat cells

- 15 Chimeric receptor constructs were sub-cloned from pBluescriptSK+ into the expression vectors pEE6hCMV.ne and pEE6hCMV.gpt (Bebbington (1991), Methods 2, 136-145) on a Hind III to EcoR I restriction fragment. The hCTMO1/CD8/ Zeta chimeric receptor construct was cloned into pEE6hCMVne and the hCTMO1 / CD8 /CD28 and hCTMO1 Zeta-CD28  
20 fusion chimeric receptor constructs were cloned into pEE6hCMVgpt.

- Plasmids were linearised and transfected into Jurkat E6.1 cells (ECACC) by electroporation using a Bio-Rad Gene Pulser using the method of Rigley *et al* (J. Immunol. (1995) 154, 1136-1145). Chimeric - receptor  
25 expressing colonies were selected in media either containing the drug G418 (2 mg/ml) for Neo vectors or Mycophenolic acid for Gpt vectors as described (Rigley *et al* ibid.). After approximately four weeks colonies were visible. Colonies were screened by analysis of surface expression of single chain Fv.  
30

#### Antibodies

- Anti-idiotype antibodies are purified antisera from rabbits immunised with hCTMO1. Anti-Id antibodies were purified initially on Protein A-Sepharose, absorbed out against human IgG-Sepharose and finally  
35 affinity purified on hCTMO1. OKT3 recognises an extracellular component of human CD3 ε (ATCC). Anti-CD28 used in these

experiments was a rat IgG2b monoclonal antibody (clone YTH 913.12) directed against the extracellular component of human CD28 (Cymbus Bioscience). FITC labelled donkey anti-rabbit Ig recognises rabbit heavy and light chains (Jackson Research Laboratories).

5

**Analysis of surface expression of scFv**

Approximately  $5 \times 10^5$  cells were stained with saturating concentrations of anti-idiotype ( $10 \mu\text{g}/\text{ml}$ ), then incubated with fluorescein-conjugated donkey anti-rabbit antibody. Fluorescence was analysed by a FACScan 10 cytometer (Beckton Dickinson).

**Anti-Id stimulation**

1  $\times 10^6$  Jurkat transfectants were incubated in a 96 well plate (Nunc) previously coated with / without a saturating concentration of anti-idiotype 15 antibody at  $37^\circ\text{C}$  / 5% CO<sub>2</sub> in non-selective media. Additional stimuli of anti-CD28 and OKT3 were added in solution to a final concentration of 5 $\mu\text{g}/\text{mL}$ . After 18 to 20 hours cells were centrifuged and supernatant assayed for human IL-2 (Quantikine kit, R & D Systems).

20 **Antigen expressing cell stimulation**

1  $\times 10^6$  Jurkat transfectants were incubated with 1  $\times 10^5$  MCF-7 cells (P.E.M. antigen expressing) in a 96 well plate (Falcon) overnight at  $37^\circ\text{C}$  / 5% CO<sub>2</sub>.

25 Additional stimulus of anti-CD28 was added in solution to a final concentration of 5 $\mu\text{g}/\text{mL}$ . After 18 to 20 hours cells were centrifuged and supernatant assayed for human IL-2 (Quantikine kit, R & D Systems).

30 **RESULTS**

Cross-linking the T-cell receptor with anti-CD3 antibodies can be used to stimulate human T-cell lines such as Jurkat E6.1 to produce cytokines including IL-2. The expression of IL-2 can be further enhanced by co-stimulation by means of antibodies to the CD28 cell surface molecule in 35 this cell line. This therefore provides a convenient model system to

evaluate chimeric receptors for the ability to deliver signals which are co-stimulatory for T-cell activation.

1. Enhancement of IL2 production by a Jurkat E6.1 cell line transfected with an hCTM01 scFv-CD8- TCR  $\zeta$  chimeric receptor (plasmid pTB3 in response to antigen or anti-idiotype antibody by co-stimulation with an anti-CD28 antibody.

The cell lines TB 3.2, 3.13 and 3.24 were stable cell lines derived from Jurkat E6.1 transfected with CTM01hscFv/CD8/Zeta. Figure 10 shows IL2 production by these cell lines when stimulated with an anti-CTMO1 idotypic antibody alone or in combination with an anti-CD28 antibody. In each case the co-stimulation with anti CD-28 results in a greater than 2-fold stimulation of IL2 production compared to stimulation with anti-CTM01 idotype antibody alone. Incubation of these cell lines with anti-CD28 alone did not result in stimulation of IL2.

Figure 11 shows the production of IL2 by one of the above cell lines (TB 3.13) when stimulated with antigen expressing tumour cells. As in figure 10 this is shown with and without co-stimulation using anti-CD28 antibody and indicates that co-stimulation can enhance IL-2 production when stimulation of the chimeric receptor is mediated by antigen.

2. Construction and testing of a chimeric receptor designed to generate a response analogous to CD28 stimulation on interaction with the extracellular scfv component.

Having established that co-stimulation via the CD28 molecule could result in enhancement of the response of a T cell transfectant to a tumour associated antigen a chimeric receptor incorporating the CD28 transmembrane and cytoplasmic components was constructed. This hCTM01/CD8/CD28 chimeric receptor (pHMF332) (HGT1) was transfected into Jurkat E6.1 cells to generate stable cell lines. Two of these lines HGT 1.2 and 1.4 were incubated in the presence of various combinations of stimulating antibodies as shown in figure 12 (see materials and methods for experimental procedure), and anti-idiotypic antibody was used to stimulate the chimeric receptor.

Incubation of the cell lines shown with an anti-CD3 antibody resulted in a low level of IL2 production. This stimulation could be enhanced by co-stimulating with an anti-CD28 antibody (column 5 figs. 12a and 12b).

- 5 Incubation with the anti-CD28 alone as expected did not result in IL2 production.

Similarly incubation with the anti-idiotypic antibody alone (stimulating the chimeric CD28 receptor) resulted in no IL2 production. However, by 10 analogy with the combined anti-CD3 and anti-CD28 stimulation, incubation with anti-CD3 and anti-idiotype resulted in IL2 production enhanced over CD3 stimulation alone. This demonstrates that a chimeric receptor could be constructed that responds via stimulation of extracellular scFv to generate an intracellular signal capable of costimulating CD3 mediated 15 activation.

3. Provision of both primary and accessory stimulation in the same effector cell.

In order to provide both primary (for example TCR  $\zeta$  mediated) and co-stimulatory (for example CD28 mediated) activation of the effector cell via interaction of a chimeric receptor with a defined ligand or antigen a fusion 20 receptor incorporating two different signalling components was constructed. This chimeric receptor hCTM01/CD8/TCRZeta-CD28 (pHMF334) was transfected into Jurkat E6.1 cells and stable lines 25 selected. One of these lines (HGT 2.4) was incubated with various combinations of antibodies and IL2 production measured (see Fig. 13).

The anti-CD3 and anti-CD28 antibodies individually and in combination 30 resulted in a similar relative stimulation of IL2 production to that seen with the other transfected cell lines. However, with the construct HGT2 the anti-idiotype antibody alone resulted in a level of IL2 production greater than achieved with the combined anti-CD3 and anti-CD28 antibodies. Furthermore, the stimulation achieved with the single anti-idiotypic 35 interaction could not be enhanced by further co-stimulation with anti-CD3, anti-CD28 or combinations of these.

**EXAMPLE 3****Analysis of single gene hP67.6-chimeric receptor constructs expressed in Jurkat cells**

In order to confirm the results obtained with the hCTMO1 fusion receptor  
5 for a different antibody scFv, and to evaluate additional fusion receptors, a  
number of different chimeras based on the hP67.6 scFv were introduced  
into Jurkat cells.

Chimeric receptor constructs hP67.6 / G1 / Zeta (HGT16), hP67.6 / G1 /  
10 Zeta-CD28 (HGT17), hP67.6 / G1 /CD28-Zeta (HGT21), hP67.6 / G1  
/CD28-Zeta-CD28 HGT26), hP67.6 /h.28 / Zeta-CD28 (HGT20) and  
hP67.6 /h.28 / CD28-Zeta (HGT22) chimeric receptor constructs were  
sub-cloned from pBluescriptSK+ into the expression vector pEE6hCMV.ne  
as described in Example 2. Expression plasmids were transfected into  
15 Jurkat E6.1 and permanent cell lines expressing chimeric receptors on  
their cell surfaces were identified as described above (Example 2) but  
using a purified rabbit anti-p67.6 idiotype antiserum prepared as described  
for hCTMO1 anti-idiotype. Alternatively, cells were stained with purified  
recombinant CD33 extracellular domain conjugated to FITC (10 µg/ml)  
20 and analysed directly on the cytometer.

Western blot analysis was carried out on representative clones for each  
construct to confirm that chimeric receptors of the expected size were  
expressed. Approximately  $10^7$  cells were lysed in lysis buffer (1%  
25 NP40, 150mM NaCl, 10mM NaF, 0.4mM EDTA, 1mM Na vanadate, 1  
mg/ml Pefabloc, 10 µg/ml Pepstatin, 10 µg/ml Leupeptin, 20 µg/ml  
Aprotinin) and samples subjected to SDS-PAGE with or without reduction  
of cystine residues with β-mercaptoethanol. Western blots were probed  
with rabbit anti-P67.6 idiotype followed by horseradish - peroxidase (HRP)  
30 conjugated donkey anti-rabbit Ig or HRP-conjugated rabbit anti-human Fc  
antisera according to standard techniques.

A comparison of the apparent molecular weights of the chimeric receptors  
in reduced and non-reduced samples indicated that the zeta-chain  
35 chimera in cell line HGT16.1 and the fusion receptor in HGT17.39 were  
present as di-sulphide linked homodimers. The CD28 chimera in HGT14.1

is present as approximately 50% disulphide-linked homodimers and approximately 50% of the molecules are not disulphide linked. At least 50% of molecules are disulphide - linked in the case of the fusion receptors in HGT20, HGT21 and HGT22 cell lines.

5

A panel of independent transfectant clones for each construct were analysed for IL-2 production in response to cells which express CD33 (HL60 cells) or are CD33 negative (eg Jurkat E6.1). It is important to analyse a number of clones expressing each construct since individual 10 clones vary substantially in the level of expression of chimeric receptor. Moreover, even clones expressing similar levels of receptor show different capacities to produce IL-2. Each transfectant was mixed with an equal number of target cells (eg  $10^5$  cells of each cell type per well of a 96-well plate) and co-cultured for approximately 20 h. The concentration of IL-2 in 15 the supernatant was then determined using a Quantikine human IL-2 ELISA (R&D Systems).

Cell lines containing construct HGT 16 produce levels of IL-2 in response to HL60 cells of up to approximately 200 pg/ml and do not produce 20 detectable IL-2 when stimulated with CD33 - negative cells. Cell lines expressing fusion receptors HGT17, 20, 21, 22 and 26 also produce IL-2, specifically in response to CD33 positive target cells, indicating that the zeta-chain signalling capacity is intact in the fusion proteins. In fact cells expressing the fusion receptors at comparable levels on the cell surface 25 produce on average more IL-2 in response to HL60 cells than HGT16 cell lines (from 50% more to 7-fold more), consistent with their capacity to provide both primary and co-stimulatory signals.

The function of the CD28 signalling domain can be confirmed by assaying 30 for recruitment of downstream signalling components to the CD28 intracellular domain in response to receptor ligand binding. The association of the regulatory (p85) sub-unit of PI3-kinase with phosphorylated ITAM motifs of the sequence YM XM (single-letter amino acid code) in the CD28 intracellular domain in response to CD28 35 stimulation is well documented (eg Stein et al., 1994 Mol. Cell. Biol. 14: 3392-3402). CD28 also associates specifically with the tyrosine kinase ITK

on activation (August et al. 1994 Proc. Natl. Acad. Sci. USA 91: 9347-9351).

Association of p85 with the receptor chimeras is analysed by  
5 immunoprecipitation of the receptor and detection of bound p85 protein by  
Western blotting as follows. Approximately  $5 \times 10^7$  cells are washed once  
with PBS and activated in 0.5 ml PBS containing 10 µg/ml rabbit anti-  
P67.6 idiotype antibody at 37°C for various times from 0 - 10 mins. Cells  
are then washed twice with ice-cold PBS and lysed in 1 ml lysis buffer as  
10 described above. Lysates are centrifuged at 15000 rpm in an Eppendorf  
micro-centrifuge for 10 min. and the supernatants immunoprecipitated with  
100 µl protein A - sepharose beads (Pharmacia) at room tempeature for  
30 min. (This immunoprecipitation procedure also serves to  
15 immunoprecipitate chimeric receptors containing antibody constant  
regions from cells which have not been stimulated with anti-idiotype  
antibody to act as a negative control). The beads are then washed 3 times  
with fresh lysis buffer, resuspended in 50 µl SDS loading buffer and  
subjected to SDS-PAGE and Western blotting. Blots are probed with  
mouse anti-p85 monoclonal antibody and HRP-conjugated rabbit anti-  
20 mouse Ig according to standard techniques.

This showed that p85 can associate with fusion receptors but not with the  
zeta chain chimera in cell line HGT16.1 thus confirming that p85  
associates specifically with CD28 and not zeta and that CD28 signalling is  
25 retained in fusion chimeras.

Association of ITK with CD28 intracellular components is detected using  
published methods (August et al. 1994 Proc. Natl. Acad. Sci. USA 91:  
9347-9351).

**EXAMPLE 4****Expression of two hP67.6 - chimeric receptors in the same cell .**

- In order to express both a zeta chimeric receptor and a CD28 co-stimulatory receptor chimera in the same cell, stably transfected Jurkat cell  
5 lines expressing CD28 receptor chimeras were infected with recombinant adenovirus encoding the hP67.6 / G1 / Zeta chimeric receptor.

- The hP67.6/h.28/CD28 construct was sub-cloned into pEE6hCMV.gpt and transffected into Jurkat E6.1 cells as described in Example 2. Cell line  
10 HGT14.1 is a Jurkat transfectant expressing this construct. The hP67.6/G1/CD28 construct was cloned into pEE6hCMV.ne and Jurkat clones HGT23.11 and HGT23.16 expressing this construct were isolated as in Example 2. The levels of expression of the CD28 chimeras on the surface of the transfected cells, determined by FAC-analysis with FITC-  
15 CD33 as described in Example 3, is shown in Figure 18.

- In order to transiently express a uniform amount of the zeta-chain chimera hP67.6/G1/ zeta in each of these CD28-chimera cell lines, a recombinant adenovirus vector expressing the zeta chimera was constructed as  
20 follows. The hP67.6/G1/zeta coding sequence from pHMF342 (Example 1 and Table 2) was excised as a Not1 - Kpn1 fragment and inserted into the adenovirus-5 transfer vector pAL119 (provided by G. Wilkinson, Department of Medicine, University of Wales, Cardiff; unpublished) between the Not1 and BamH1 sites, after insertion of a Kpn1 - BamH1  
25 adaptor oligonucleotide, to form pAL119-342. In this plasmid, the chimeric receptor coding sequences are expressed under the control of the hCMV-MIE promoter-regulatory region and polyadenylation signal (Wilkinson and Akrigg 1992 Nucl. Acids Res.20: 2233-2239).

- 30 Suitable alternative adenovirus transfer vectors containing the hCMV-MIE promoter include pCA3 and pCA4 (Hitt et al. 1995 in Methods in Molecular Genetics, K.W. Adolph (ed) Academic Press, Orlando.) Alternative adenovirus transfer vectors can be used such as pAC (Gerard and Meidell 1995 In DNA Cloning: a practical approach (2nd edition) Volume 4 ed  
35 Glover and Hames, IRL Press) which does not contain a promoter. In this case, one of many other heterologous promoters, such as the RSV-LTR

promoter or T-cell specific promoters, may be introduced upstream of the chimeric receptor coding sequence prior to insertion into the transfer vector. Additional RNA processing signals are also desirable, such as a polyadenylation signal (eg from SV40 Virus) and an intron (e.g. from the hCMV-MIE gene) (Bebbington (1991), Methods 2, 136-145).

Approximately 5 µg pAL119-342 was co-transfected with 5µg pJM17 (Microbix Biosystems Inc., McGrory et al. 1988 Virology 163: 614-617) into the human embryonic kidney cell line, 293 (ATCC CRL 1573) by calcium phosphate-mediated transfection, according to standard procedures for construction of adenovirus recombinants (Lowenstein et al 1996 in Protocols for gene transfer in Neuroscience, P.R. Lowenstein and L.W. Enquist (eds) Wiley and Sons). This generated recombinant virus RAd160 containing the chimeric receptor cDNA under the control of hCMV - MIE gene regulatory regions. Large scale preparations of RAd160 were prepared (Lowenstein et al ibid.) with titres of greater than  $10^{10}$  pfu/ml and stored at -70°C in small aliquots.

Recombinant adenoviruses containing coding sequences for CD28 chimeric receptors are prepared in the same way after insertion of the desired coding sequence into pAL119 or another adenovirus transfer vector.

RAd160 was added to Jurkat E6.1 cells or transfectants expressing CD28 receptor -chimeras at a multiplicity of infection (MOI) of up to 400 pfu/cell with 2 µg/ml DEAE - Dextran and incubated for 24h at a cell concentration of  $10^6$  cells/ml in the presence of virus. Samples of cells were infected with a recombinant adenovirus expressing an irrelevant β-galactosidase protein RAd35 (Wilkinson and Akrigg 1992 Nucl. Acids Res.20: 2233-2239) in the same way to act as a negative control. Infected cells were then washed once in fresh growth medium, expanded in culture for a further 6 days and assayed for IL-2 production in response to target cells. The results are shown in Figure 19. Jurkat cells infected with RAd160 produce essentially undetectable levels of IL-2 in response to HL60-cell stimulation (less than 10 pg/ml) unless co-stimulated with 10 µg/ml anti-CD28 antibody 15E8 (Caltag) which leads to low levels of IL-2 production

specifically in response to HL60 cells and not in response to a cell line which does not express human CD33, the murine SP2/0 cell line. In contrast, RAd160-infected HGT14.1 cells, which express a CD28 chimeric receptor, produce significant levels of IL-2 specifically in response to HL60 target cells even in the absence of anti-CD28 antibody. This indicates that the CD28-chimeric receptor hP67.6/h.28/CD28 is able to contribute the requisite co-stimulation to the zeta chimera. Cell lines expressing the alternative CD28 chimeric receptor, hP67.6/G1/CD28, 23.11 and 23.16 show markedly reduced levels of IL-2 production compared with 14.1.

Indeed, 23.16, the cell line expressing the highest level of this CD28 chimera produces no detectable IL-2 at all. The CD28 signalling pathway was shown to be intact in this cell line since stimulation through CD3 (using anti-CD3 antibody) in 23.16 yields very high levels of IL-2 (results not shown). Thus the signalling defect in cell lines expressing the hP67/G1/CD28 chimera appears to be due to interference with zeta-chain signalling. The mechanism responsible is likely to be related to the use of the same extracellular domain in the zeta and CD28 chimeric receptors. This will allow heterodimerisation of the two receptors and this appears to interfere with zeta-chain signalling. This hypothesis is supported by the fact that 23.16, expressing high levels of the CD28 chimera, shows greater interference with zeta-chain signalling than 23.11, expressing very low levels of the CD28 chimera (Figure 18).

This experiment shows that it is possible to use the same scFv region to stimulate two chimeric receptor molecules in the same cell, one to provide a primary stimulus in response to antigen and the other receptor to provide a co-stimulatory signal. This leads to efficient IL-2 production specifically in response to antigen - expressing target cells provided that the two receptors are prevented from heterodimerisation, for instance by using different dimerisation domains on the two receptors. It is envisaged that additional pairs of dimerisation domains will be compatible. For instance the scFv/h.28/zeta chimeric receptor (Example 1; Figure 17) could provide the primary signal and the scFv/G1/CD28 receptor (Example 1; Figure 16) would provide the co-stimulatory signal.

**EXAMPLE 5****Identification of additional co-stimulatory cell-surface receptors using anti-receptor antibodies.**

5  $5 \times 10^5$  HGT16.1 cells expressing the hP67.6 scFv/G1/zeta chimeric receptor (Example 3) were incubated for 16h with an equal number of HL60 cells in the presence of various mouse monoclonal antibodies directed against human T-cell surface markers. The bivalent antibodies were included at 10 µg/ml to test for their ability to co-stimulate the zeta - chain chimera. The antibodies used in this experiment were: anti-CD2  
10 RPA2.10 (Pharmingen), anti-CD3 OKT3 (ATCC), anti-CD4 OKT4 (ATCC), anti-CD5 UCHT2 (Pharmingen), anti-CD28 15E8 (Caltag) and a control antibody MOPC21 (ATCC). IL-2 accumulated in the supernatant at the end of the incubation was measured by Quantikine IL-2 ELISA (R&D Systems).

15

The results indicate that anti-CD2, anti-CD5 and anti-CD28 co-stimulate production of IL-2 in HGT16.1 cells in response to HL60 target cells hence confirming CD2, CD5 and CD28 as co-stimulatory receptors compatible with zeta-chain signalling. From experiments designed in this  
20 way, it would be possible to determine the co-stimulatory activity of other cell surface molecules. The intracellular domains can then be included in chimeric receptors as described in Example 1 and evaluated as described in Examples 2, 3 and 4.

25 **EXAMPLE 6**

**Introduction of chimeric receptors into primary human CTLs.**

In order to establish an assay for co-stimulation of cytolytic T-cell function, a zeta-chain chimera was introduced into primary human T-cells using recombinant adenovirus vectors. Peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteers using centrifugation over Ficoll-Hypaque (Pharmacia) according to the manufacturer's instructions and cultured in RPMI-1640 medium with 10% FCS in 175-cm<sup>2</sup> tissue culture flasks. Non-adherent cells were transferred to fresh tissue culture flasks after 24h and phytohaemagglutinin (PHA) was added to a final concentration of 2 µg/ml and human recombinant IL-2 at 50ng/ml. After 6 days, CD4 - positive cells were removed using anti-CD4 antibody

immobilised on magnetic Dynabeads (Becton - Dickinson) to leave a population of cells at least 95% CD8 - single positive (CTL cells). The cells were washed by centrifugation and resuspended in fresh medium +10% FCS at  $10^6$  cells /ml.

5

Recombinant adenovirus RAd160 (expressing the hP67.6/G1/zeta chimeric receptor, Example 4) or the control virus RAd35 was added to the cells at a multiplicity of infection (MOI) of up to 400 pfu/cell with 2  $\mu$ g/ml DEAE-Dextran and incubated for 24h. Samples of cells were then fixed in

- 10 1% glutaraldehyde in PBS and infection rates measured by staining RAd35 - infected cells for  $\beta$ -galactosidase activity using 5-Bromo-4-chloro-3-indolyl  $\beta$ -D-galactoside (X-gal; Promega, according to the manufacturer's instructions). By this method, infection frequencies were determined to be at least 80%. Infected cells were expanded in culture for  
15 15 a further 6 days in medium containing 50 ng/ml human IL-2. In some experiments, 2mM sodium butyrate was added to infected CTL cells to induce expression from the hCMV-MIE promoter.

Cytolytic activity against the CD33-expressing tumour cell line HL60 was

- 20 detected in recombinant adenovirus - infected CD8-positive cells incubated for 6 days in IL-2 and 2mM butyrate using standard 6h  $^{51}\text{Cr}$  release assays.  $2 \times 10^7$  HL60 target cells were labelled by incubation with 25MBq  $^{51}\text{Cr}$  (CJS4 Amersham) for 45 min. at 37°C in T-cell growth medium. After washing,  $1.5 \times 10^4$  labelled HL60 cells were transferred  
25 into each well of a 96-well microtitre plate in the presence of RAd - infected CD8-positive effector cells at ratios in the range 100 to 0.1 effector:target cells. Cells were incubated for 6h in T-cell growth medium before centrifuging the plates and removal of the supernatant for counting. Cytolysis was expressed as the amount of  $^{51}\text{Cr}$  released into the medium  
30 compared to that released by detergent treatment of target cells. In the experiment illustrated (Figure 20) specific lysis was mediated by RAd 160 - infected effector cells but not by CD8-positive cells infected with RAd35. The degree of specific lysis is increased with increased E:T ratio.  
35 This assay is useful for determining the effects of co-stimulation on cytolytic function using anti-receptor antibodies, co-stimulatory cytokines

or co-stimulatory chimeric receptors. Cells starved of IL-2 for various lengths of time can also be used to increase the sensitivity of assays designed to evaluate co-stimulatory activities. CD28 chimeric receptors can be introduced by co-infection of recombinant adenovirus with RAd160.

- 5 Alternatively a fusion receptor containing both zeta and CD28 signalling domains can be introduced using a single recombinant adenovirus. Anti-receptor antibodies which may be screened in this assay include anti-CD2 and anti-CD5 (see Example 5).

10 **EXAMPLE 7**

**Analysis of co-stimulatory activities in Macrophages and Monocytes.**

Human monocytes were isolated from peripheral blood as follows. PBMC were isolated as described above and adherent cells obtained by settling on to plastic tissue culture flasks for 24 h before washing extensively with

15 fresh medium.

Primary macrophages were isolated from the peritoneal cavity of Wistar rats 5 days after i.p. injection of 5 ml 3% thioglycollate (Sigma T-9032) in saline according to the method of Argys (Argys 1967, J.Immunol. 99:744-

20 750) or 3 ml mineral oil (heavy white oil; Sigma 400-5). Peritoneal lavage was carried out with 20ml RPMI 1640 medium + 10% FCS and 3.15% sodium citrate. Greater than 60% of the cells in the peritoneal lavage were mononuclear phagocytes as defined by flow cytometry using FITC-conjugated mouse anti-rat macrophage antibody ED2 (Serotec) and

25 morphological characteristics. Adherent cells were enriched by applying cells to plastic flasks or 6-well plates in RPMI 1640 medium + 10% FCS and culturing for 2 days. Non-adherent cells were then removed by extensive washing with fresh medium. Alternatively, macrophages were purified by Percoll density centrifugation (Lawson and Stevenson 1983 Br.

30 J. Cancer 48: 227-237.)

Monocytes and macrophages were maintained in culture for 48h and infected with recombinant adenoviruses at a MOI of up to 200 pfu/cell for 16h in the presence of 2 µg/ml DEAE-Dextran, after which the virus was

35 removed by washing with fresh medium. Up to 80% of human peripheral-blood monocytes and rat peritoneal macrophages were infectable using

this procedure, as determined using X-gal staining of cells infected with RAd35. The use of higher concentrations of virus increased the percentage of cells infected but led to a significant reduction in cell viability.

5

The recombinant adenovirus RAd160 can be used to provide a human CD33-specific primary stimulus to cells of the rat or mouse monocyte - macrophage lineage. Since human monocytes express the CD33 antigen, for the analysis of chimeric receptor function in human monocytic

10 phagocytes, it may be more appropriate to use an alternative binding specificity such as the hCTMO1scFv - containing chimeric receptor, constructed as in Example 1 and inserted into a recombinant adenovirus vector. Additionally, the zeta chain sequences of the chimeric receptor may be substituted with the transmembrane and intracellular domain of a

15 FcRIII  $\gamma$  chain (Park et al 1993, J. Clin. Invest. 92: 2073-2079).

Rat peritoneal macrophages infected with RAd160 at an MOI of 100 pfu/cell, expressed high levels of chimeric receptor on their surfaces 48h post-infection as determined by staining with FITC-CD33 and analysis by

20 a FACScan flow cytometer.

The response of monocytes and macrophages expressing the appropriate chimeric receptor to stimulation with specific antigen or antigen-expressing cells recognised by the scFv is measured in standard  $^{51}\text{Cr}$  release assays

25 (Example 6). Alternatively, phagocytosis and cytostasis assays (Lawson and Stevenson 1983 Br. J. Cancer 48: 227-237) or assays for the release of cytokines are carried out eg human TNF ELISA (R&D Systems) or rat TNF ELISA (Biosource).

30 Identification of appropriate receptor intracellular domains to provide a co-stimulatory signal can be accomplished by incubation of macrophages expressing the chimeric receptor with a source of the specific antigen and with cross-linking antibodies or natural ligands specific for individual cell surface receptors present on monocytes and macrophages as described

35 in Example 5. Suitable receptors include the IL-2 receptor, the CSF-1 receptor, the IFN- $\gamma$  receptor, the GM-CSF receptor and TNF receptors.

- Natural ligands which can be used for human monocytes / macrophages include recombinant human IL-2, human CSF-1 (M-CSF), human IFN $\gamma$ , human GM-CSF and human TNF $\alpha$  (all from Genzyme). Ligands which can be used for rat or mouse macrophages include recombinant rat or human 5 IL-2, human CSF-1 (M-CSF), mouse IFN $\gamma$ , mouse GM-CSF and mouse TNF $\alpha$  (Genzyme). Species-specific antibodies which cross-link and stimulate the chosen receptors can be raised using standard techniques or can be identified by screening commercially available antibodies.
- 10 Those antibodies or natural ligands which co-stimulate macrophage responses to CD33 identify candidate receptors whose intracellular domains or associated signalling molecules, such as receptor - associated tyrosine kinases, can be used to produce chimeric co-stimulatory receptors or fusion receptors containing both co-stimulatory and primary 15 signalling domains as described in Example 1. The intracellular components which may be used in these chimeric receptors include the following. The intracellular domains of the GM-CSF receptor  $\beta$  chain can be used as part of a di-sulphide linked homodimeric receptor or in combination with an intracellular component from the  $\alpha$  chain (Muto et al. 20 1996, J. Exp. Med. 183: 1911-1916). The intracellular domains of the IFN $\gamma$ -receptor  $\alpha$  and  $\beta$  chains can be used (Bach et al., 1996.. Mol. Cell. Biol. 16: 3214-3221.), as can the intracellular domains of the IL-2 receptor, particularly the  $\beta$  and  $\gamma$  chains . One or more intracellular tyrosine kinase 25 components can be used such as the jak1, jak2 and jak3 kinases or the intracellular domain of the CSF-1 receptor tyrosine kinase (Carlberg and Rohrschneider 1994 Mol. Biol. Cell 5:81-95). If these tyrosine kinases are used, the receptors containing them are preferably constructed so that they are presented on the cell surface as monomers which oligomerise on binding of the scFv component to the target antigen, for instance using a 30 scFv coupled to a CD8 hinge extracellular component , coupled to a CD28 transmembrane component (see Example 1) which is coupled to the tyrosine kinase component.

**EXAMPLE 8**

- 35 Analysis of co-stimulatory activities in other cells of the immune system

- Additional immune cell types such as CD4-positive T-cells, B-cells, NK cells, basophils, neutrophils, haematopoietic stem cells are isolated from human peripheral blood, mouse or rat blood or peritoneal cavity or other sources by published procedures (Current Protocols in Immunology ed 5 Coligan et al. John Wiley and Sons). Established cell lines which retain the differentiated functions of various immune cell types can also be used eg the human NK-like cell line YT2C2 (Roger et al 1996 Cellular Immunol. 168: 24-32.) A chimeric receptor capable of delivering a primary stimulus such as the hP67.6/G1/zeta chimera described above is introduced into 10 the isolated immune cell type, eg by infection with recombinant adenovirus RAd160, and cross-linking antibodies or natural ligands of cell surface receptors are used to identify cell-surface molecules capable of providing co-stimulatory signals as described in Example 7.
- 15 Chimeric receptors containing appropriate cytoplasmic components to provide suitable co-stimulatory functions are then constructed as described in Example 1. The function of the chimeric receptors in the chosen cell types can be analysed using recombinant adenovirus vectors.

## POSSIBLE CHIMERIC RECEPTOR COMBINATIONS

TABLE 1

	LIGAND BINDING	SPACER	TRANS MEMBRANE	SPACER	CYTOSOLIC COMPONENT	SPACER	CYTOSOLIC COMPONENT	SPACER	CYTOSOL SPACERS
A	TAA SCFV	G1	TCR ZETA	OPT**	TCR ZETA	OPT	OPT	OPT	OPT
	TAA SCFV	h	CD28	OPT	CD28	OPT	OPT	OPT	OPT
B	TAA SCFV	CD8	TCR ZETA	OPT	TCR ZETA	OPT	OPT	OPT	OPT
	TAA SCFV	h	CD28	OPT	CD28	OPT	OPT	OPT	OPT
C	TAA SCFV	G1	TCR ZETA	OPT	TCR ZETA	OPT	OPT	OPT	OPT
	TAA SCFV	G1	IL2 R β	OPT	IL2 R β	OPT	IL2 R γ	OPT	OPT
D	TAA SCFV	G1	TCR ZETA	OPT	TCR ZETA	OPT	CD28	OPT	OPT
E	TAA SCFV	h	TCR ZETA	OPT	TCR ZETA	OPT	CD28	OPT	OPT
F	TAA SCFV	G1	TCR ZETA	OPT	TCR ZETA	OPT	IL2 R β	OPT	IL2 R γ

A,B and C describe pairs of genes coding for pairs of chimeric receptors

D,E and F describe fusion chimeric receptors, as shown in C one of a pair of receptors may be a fusion receptor

TAA SCFV denotes a single chain Fv to a Tumour associated antigen

For a pair of chimeric receptors the SCFVs may bind the same or different epitopes of the same antigen or different antigens on the same or different cells.

G1 is the IgG CH3 CH2 HINGE spacer construct described in the text

h denotes the IgG hinge plus part of the CD28 extracellular component described in the text

\* one or more further cytosolic and/or spacer components

\*\* OPT = optional

**TABLE 2**

5

**CHIMERIC RECEPTOR CONSTRUCTS AND CELL LINE  
NOMENCLATURE**

10	CONSTRUCT	CONSTRUCTION PLASMID	EXPRESSION PLASMID	CELL LINES
15	hCTMO1 scFv / CD8 / TCR zeta	pBS3	pTB3	TB3
	hP67.6 scFv / CD8 / TCR zeta	pBS5	pTB5	TB5
	hCTMO1 scFv / CD8 / CD28	pHMF 320	pHMF 332	HGT 1
20	hCTMO1 scFv / CD8 / TCR zeta-CD28	pHMF 326	pHMF 334	HGT 2
	hP67.6 scFv / G1 / TCR zeta	pHMF 342	pHMF 351	HGT 6 & 16
25	hP67.6 scFv / G1 / TCR zeta-CD28	pHMF 354	pHMF 355	HGT 7 & 17
	hP67.6 scFv / h / CD28	pHMF 350	pHMF 353	HGT 8 & 14
	hP67.6 scFv / G1 / CD28	pHMF 375	pHMF 376	HGT 23
30	hP67.6 scFv / G1 / CD28-TCR zeta	pHMF 372	pHMF 373	HGT 21
	hP67.6 scFv / G1 / CD28-TCR zeta-CD28	pHMF 379	pHMF 380	HGT 26
35	hP67.6 scFv / h.28 / TCR zeta	pHMF 377	pHMF 378	HGT 24
	hP67.6 scFv / h.28 / TCR zeta - CD28	pHMF 363	pHMF 364	HGT 20
	hP67.6 scFv / h.28 / CD28 - TCR zeta	pHMF 369	pHMF 371	HGT 22

40

G1 is the IgG hinge CH2 CH3 spacer

45

h is the IgG hinge component plus part of CD28 extracellular domain spacer.

h.28 is the IgG hinge component plus part of CD28 extracellular domain and amino acid residues L, D, P & K spacer.

50

Expression plasmids pTB3 and pTB5, pHMF 334, 351, 355, 378 and 364 include the TCR zeta transmembrane domain.

Expression plasmids pHMF 332, 353, 376, 373, 380 and 371 include the CD28 transmembrane domain.

55

**CLAIMS**

1. A method of activating a cell as a result of one type of extracellular interaction between said first cell and a molecule associated with a second target cell characterised in that said first cell is provided with a DNA delivery system comprising DNA coding for one or more recombinant chimeric receptors comprising two or more different cytoplasmic signalling components, wherein said cytoplasmic components are not naturally linked, and at least one is derived from a membrane spanning polypeptide.
2. A method according to Claim 1 wherein the cytoplasmic signalling components are capable of acting together cooperatively.
- 15 3. A method according to Claim 1 or Claim 2 wherein said DNA additionally codes for signal peptide, binding and/or transmembrane components of said one or more chimeric receptors, wherein the binding component is capable of recognising a cell surface molecule on a target cell.
- 20 4. A method according to Claim 3 wherein the signal peptide, transmembrane and cytoplasmic signalling components and all or part of the binding component are coded for by a single DNA coding sequence.
- 25 5. A method according to Claim 3 wherein each cytoplasmic signalling component is coded for by a separate DNA coding sequence, each of DNA sequence additionally coding for a signal peptide, a transmembrane component and all or part of a binding component.
- 30 6. A method according to Claim 4 or Claim 5 wherein said DNA codes for part of said binding component and an additional separate DNA coding sequence codes for the remainder of the binding component.
- 35 7. A method according to Claim 5 or Claim 6 wherein the binding component coded for by one DNA sequence is capable of

participating in the same type of extracellular binding event as the binding component coded for by any other DNA sequence.

8. A method according to Claim 7 wherein each binding component  
5 binds to the same molecule associated with the target cell.
9. A method according to Claim 8 wherein each binding component is  
the same.
10. 10. A method according to any one of Claims 1 to 9 wherein the one or  
more recombinant chimeric receptors are capable of recognising a  
viral or cell surface molecule on a target cell.
11. A DNA delivery system comprising DNA in association with a carrier  
15 said DNA coding for a recombinant chimeric receptor capable of one  
type of extracellular interaction and comprising two or more different  
cytoplasmic signalling components which are not naturally linked, and  
wherein at least one of said cytoplasmic components is derived from  
a membrane spanning polypeptide.  
20
12. A DNA delivery system comprising DNA in association with a carrier  
said DNA coding for two or more recombinant chimeric receptors  
each capable of the same one type of extracellular interaction and  
wherein each of said receptors comprises one or more different  
25 cytoplasmic signalling components which are not naturally linked, and  
wherein at least one of said cytoplasmic components is derived from  
a membrane spanning polypeptide.
13. A DNA delivery system according to Claim 11 wherein said DNA  
30 codes in reading frame for:
  - i) a signal peptide component;
  - ii) a binding component capable of recognising a cell surface  
molecule on a target cell;
  - iii) a transmembrane component;
  - iv) 35 two or more different cytoplasmic signalling components which  
are not naturally linked, and wherein at least one of said cytoplasmic

components is derived from a membrane spanning polypeptide; and optionally

- v) one or more spacer regions linking any two or more of said i) to iv) components.

5

- 14. A DNA delivery system according to Claim 11 wherein said DNA comprises 1) a first DNA which codes in reading frame for:
  - i) a signal peptide component;
  - ii) part of a binding component;
  - 10 iii) a transmembrane component;
  - iv) two or more cytoplasmic signalling components which are not naturally linked, and wherein at least one of said cytoplasmic components is derived from a membrane spanning polypeptide; and optionally
  - 15 v) one or more spacer regions linking any two or more of said i) to iv) components; and 2) a second separate DNA which codes in reading frame for a signal peptide component and a further part of the binding component ii) coded for by said first DNA, such that the binding component parts together are capable of recognising a cell surface molecule on a target cell.
  - 20
- 15. A DNA delivery system according to Claim 12 wherein said DNA comprises a first and a second separate DNA each of which codes in reading frame for:
  - i) a signal peptide component;
  - 25 ii) a binding component capable of recognising a cell surface molecule on a target cell;
  - iii) a transmembrane component;
  - iv) one or more different cytoplasmic signalling components which are not naturally linked, and wherein at least one of said cytoplasmic components is derived from a membrane spanning polypeptide; and optionally
  - 30 v) one or more spacer regions linking any two or more of said i) to iv) components; provided that said first DNA codes for at least one signalling component iv) that is not coded for by said second DNA.
  - 35

16. A DNA delivery system according to Claim 12 wherein said DNA comprises 1) a first and a second separate DNA each of which codes in reading frame for:
  - i) a signal peptide component;
  - 5 ii) one part of a binding component;
  - iii) a transmembrane component;
  - iv) one or more different cytoplasmic signalling components which are not naturally linked, and wherein at least one of said cytoplasmic components is derived from a membrane spanning polypeptide; and

10 optionally

  - v) one or more spacer regions linking any two or more of said i) to iv) components; provided that said first DNA codes for at least one signalling component iv) that is not coded for by said second DNA; and 2) a separate third and fourth DNA each of which codes in reading frame for a signal peptide component and a further part of the binding component ii) coded for by said first and second DNA respectively, such that the binding component parts together provided by the first and third DNA and together provided by the second and fourth DNA are each capable of recognising a cell surface molecule on a target cell.

15

20

  - 17. A DNA delivery system according to Claims 13 to 16 wherein each signal peptide component is an immunoglobulin signal sequence.
  - 25 18. A DNA delivery system according to Claims 15 to 17 wherein the binding component coded for by said first DNA is the same as the binding component coded for by said second DNA.
  - 19. A DNA delivery system according to Claims 13 to 18 wherein the 30 binding component is an antibody or an antigen binding fragment thereof.
  - 20. A DNA delivery system according to Claim 19 wherein the antibody or fragment thereof is an engineered human antibody or antigen binding fragment thereof.

21. A DNA delivery system according to Claims 18 to 20 wherein the binding component is a single chain Fv fragment.
22. A DNA delivery system according to Claims 18 to 20 wherein the binding component is a Fab' fragment.  
5
23. A DNA delivery system according to any one of Claims 13 to 22 wherein the transmembrane component is derived from all or part of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD8, CD4, 10 a cytokine receptor or a colony stimulating factor receptor.
24. A DNA delivery system according to Claim 23 wherein the transmembrane component is derived from all or part of CD28.
- 15 25. A DNA delivery system according to any one of Claims 11 to 24 wherein the cytoplasmic signalling components are capable of acting together cooperatively.
- 20 26. A DNA delivery system according to any one of Claims 13 to 25 wherein the cytoplasmic signalling components are derived from all or part of the cytoplasmic domains of a zeta, eta or epsilon chain of the T-cell receptor, CD28, the  $\gamma$  chain of a Fc receptor, a cytokine receptor, a colony stimulating factor receptor, a tyrosine kinase or an adhesion molecule, B29, MB-1, CD3 delta, CD3 gamma, CD5 or 25 CD2.
27. A DNA delivery system according to Claim 26 wherein the cytoplasmic signalling components are ITAM containing cytoplasmic components.  
30
28. A DNA delivery system according to Claim 26 or Claim 27 wherein the cytoplasmic signalling components are derived from all or part of CD28 and/or the zeta chain of the T-cell receptor.

29. A DNA delivery system according to any one of Claims 11 to 28 wherein the cytoplasmic signalling components are in any orientation relative to one another.
- 5    30. A DNA delivery system according to any one of Claims 13 to 29 wherein said DNA coding for components i) to iv) additionally codes for one or more spacer regions linking the binding component ii) and the transmembrane component iii).
- 10    31. A DNA delivery system according to Claim 30 wherein two or more different spacer regions link the binding component ii) and the transmembrane component iii), both regions either being coded for by one DNA sequence or when a first and second DNA sequence is present one region being coded for by said first DNA and the other different region being coded for by said second DNA.
- 15    32. A DNA delivery system according to Claims 30 or Claim 31 wherein the spacer region is selected to provide one or more free thiol groups.
- 20    33. A DNA delivery system according to Claims 30 to 32 wherein the spacer region is derived from all or part of the extracellular region of CD8, CD4 or CD28 .
- 25    34. A DNA delivery system according to Claims 30 or Claim 32 wherein the spacer region is all or part of an antibody constant region.
- 30    35. A DNA delivery system according to Claims 30 to 32 wherein the spacer region is derived from all or part of an antibody hinge region linked to all or part of the extracellular region of CD28.
36. A DNA delivery system according to any one of Claims 11 to 35 wherein the carrier is a viral vector or a non-viral vector.
- 35    37. A DNA delivery system according to Claim 36 wherein the non-viral vector is a liposomal vector.

38. A DNA delivery system according to Claim 37 wherein the carrier is a targeted non-viral vector.
- 5 39. A DNA delivery system according to Claim 38 wherein the targeted vector is an antibody targeted liposome.
40. A DNA delivery system according to Claim 38 wherein the targeted vector is an antibody targeted condensed DNA.
- 10 41. A DNA delivery system according to Claim 40 wherein the targeted vector is an antibody targeted protamine or polylysine condensed DNA.
- 15 42. A DNA delivery system according to Claim 38 wherein the targeted vector is antibody targeted naked DNA.
43. A DNA delivery system according to Claims 39 to 42 wherein the antibody is a whole antibody or an antigen binding fragment thereof.
- 20 44. A DNA delivery system according to Claim 43 wherein the antibody is an engineered human antibody or an antigen binding fragment thereof.
- 25 45. An effector cell transfected with a DNA delivery system according to any one of Claims 1 to 44.
46. An effector cell according to Claim 45 which is a lymphocyte, a dendritic cell, a B-cell, a haematopoietic stem cell, a macrophage, a monocyte or a NK cell.
- 30 47. An effector cell according to Claim 46 which is a cytotoxic T-lymphocyte.
- 35 48. A DNA delivery system according to any one of Claims 11 to 47 for use in the treatment of infectious disease, inflammatory disease,

cancer, allergic/atopic disease, congenital disease, dermatologic disease, neurologic disease, transplants and metabolic/idiopathic disease.

5    49. A DNA delivery system according to Claim 48 for use in the treatment of rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, asthma, eczema, cystic fibrosis, sickle cell anaemia, psoriasis, multiple sclerosis, organ or tissue transplant rejection, graft-versus-host disease or diabetes.

10

50. A pharmaceutical composition comprising a DNA delivery system according to any one of Claims 11 to 44 together with one or more formulatory agents.

15

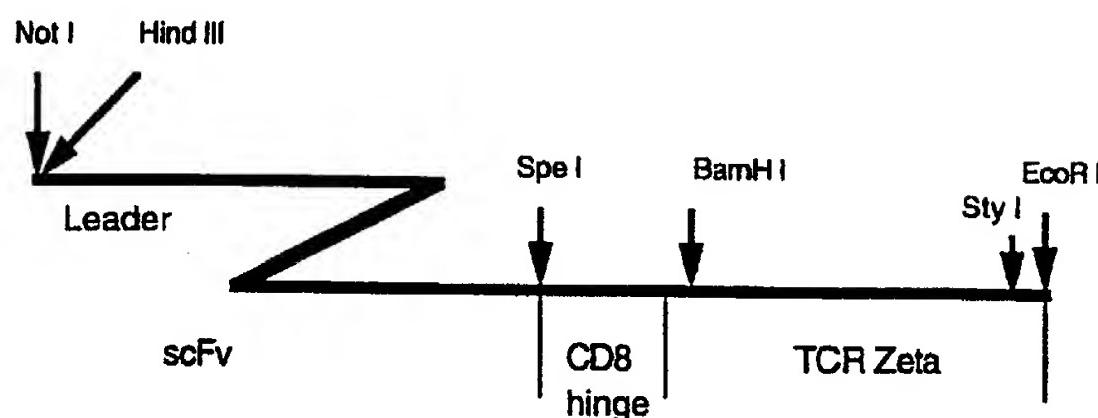
51. A pharmaceutical composition according to Claim 50 wherein the formulatory agent is a suspending, preservative, stabilising and/or dispersing agent.

20

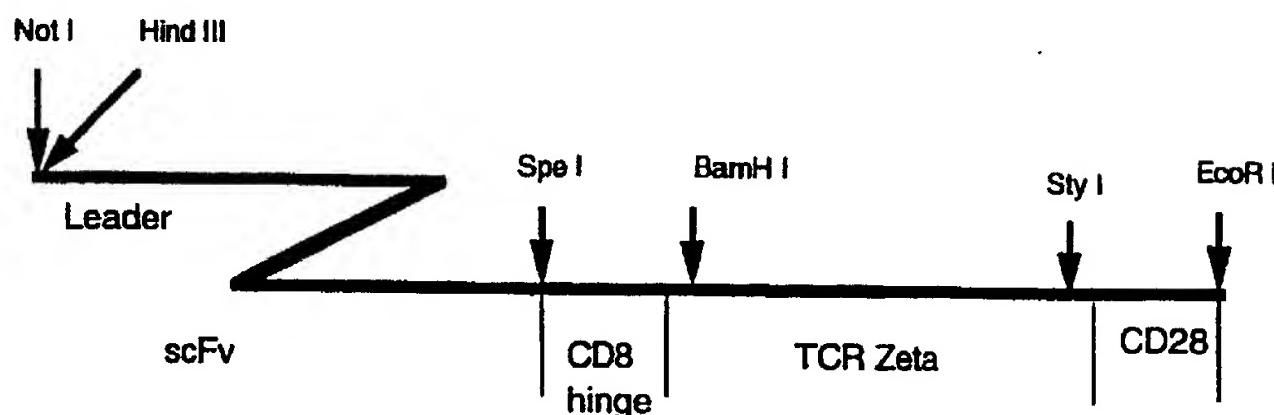
52. DNA coding for a recombinant chimeric receptor for use in a delivery system according to any one of Claims 11 to 44.

**FIG. 1**  
Construct cassettes cloned into pBluescript SK +

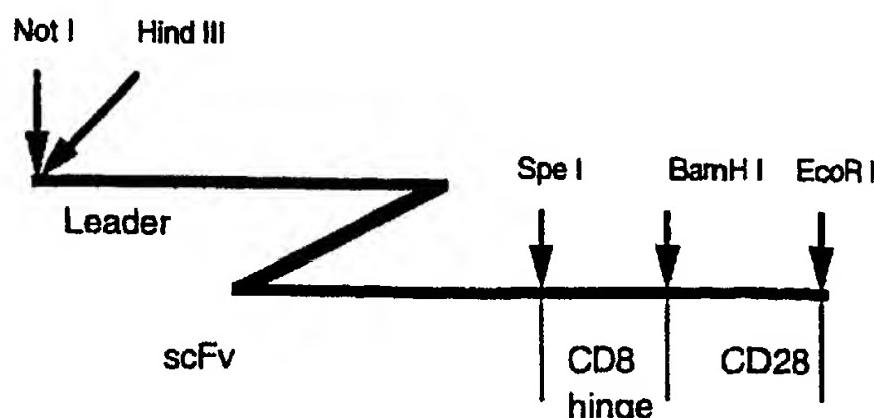
scFv / CD8 / Zeta Chimeric Receptor



scFv / CD8 / Zeta-CD28 fusion Chimeric Receptor

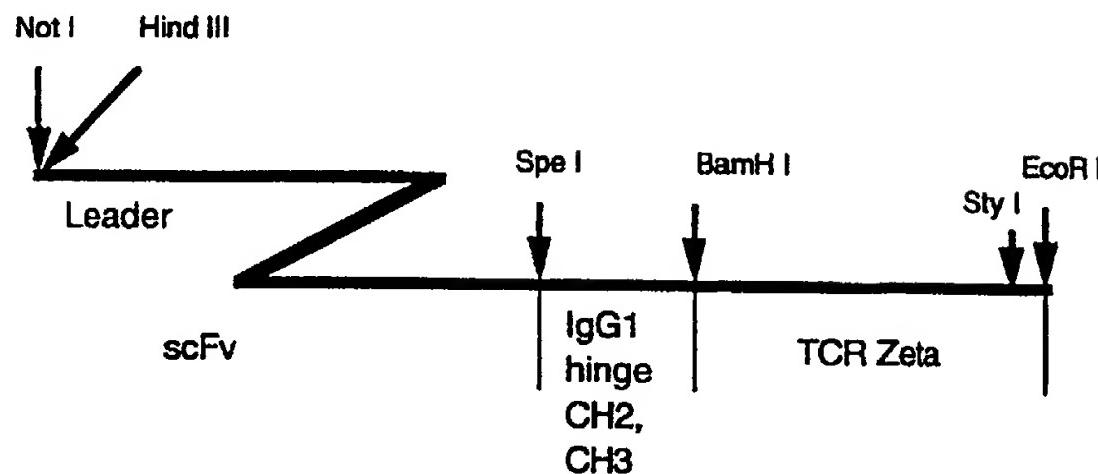


scFv / CD8 / CD28 Chimeric Receptor

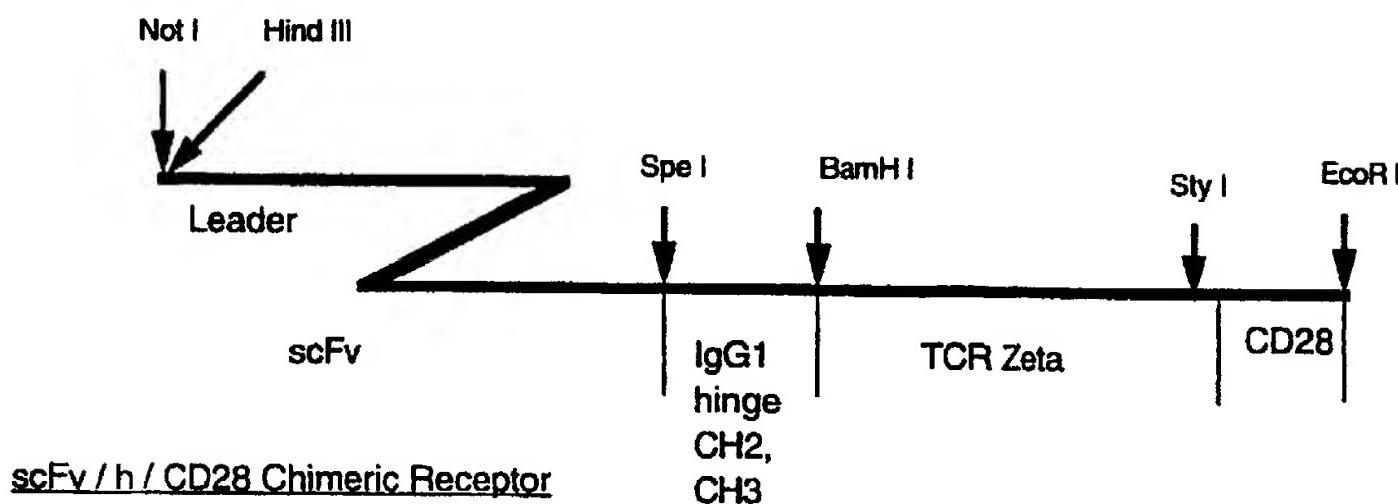


**FIG. 2a**  
Construct cassettes cloned into pBluescript SK +

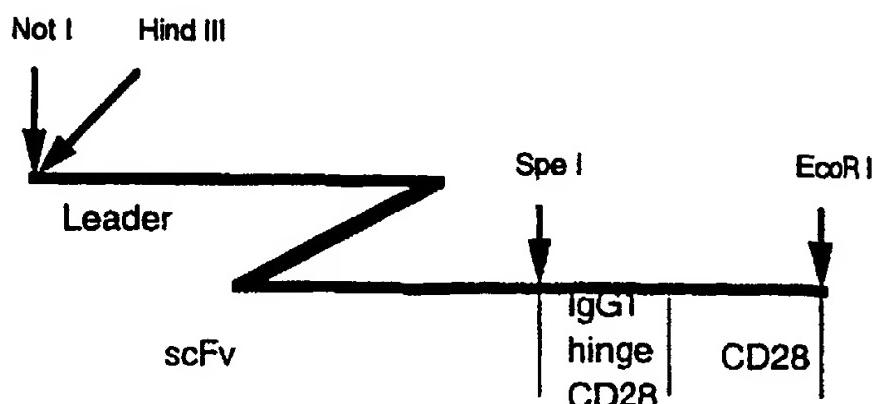
scFv / G1 / Zeta Chimeric Receptor



scFv / G1 / Zeta-CD28 fusion Chimeric Receptor



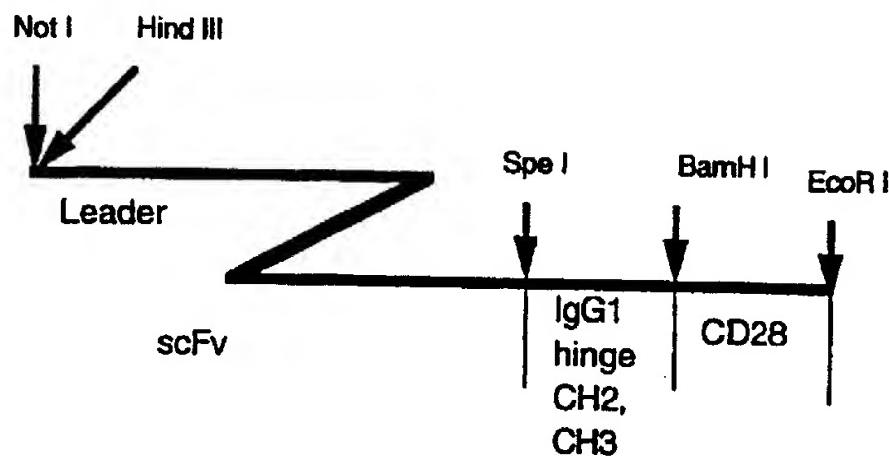
scFv / h / CD28 Chimeric Receptor



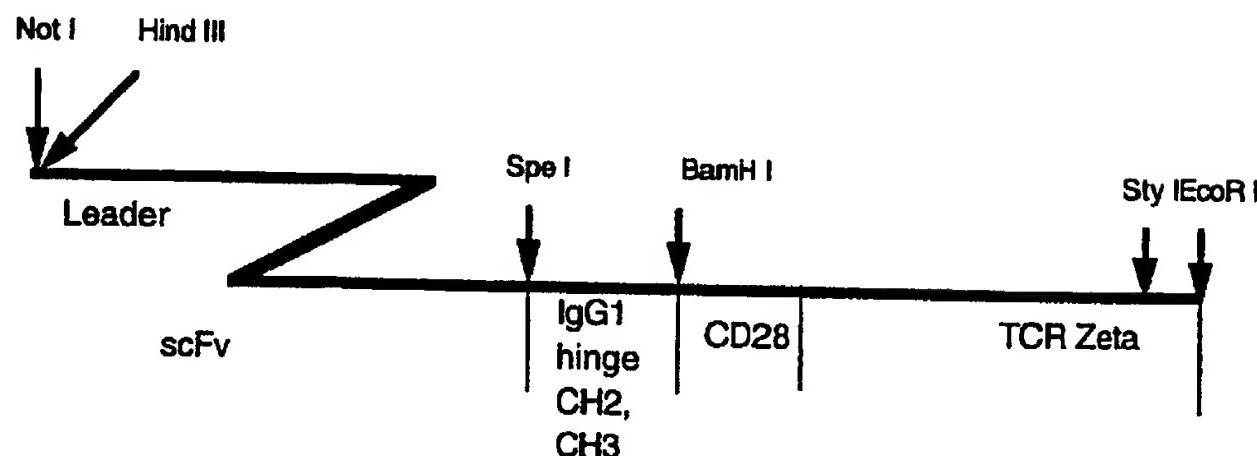
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**FIG. 2b**  
Construct cassettes cloned into pBluescript SK +

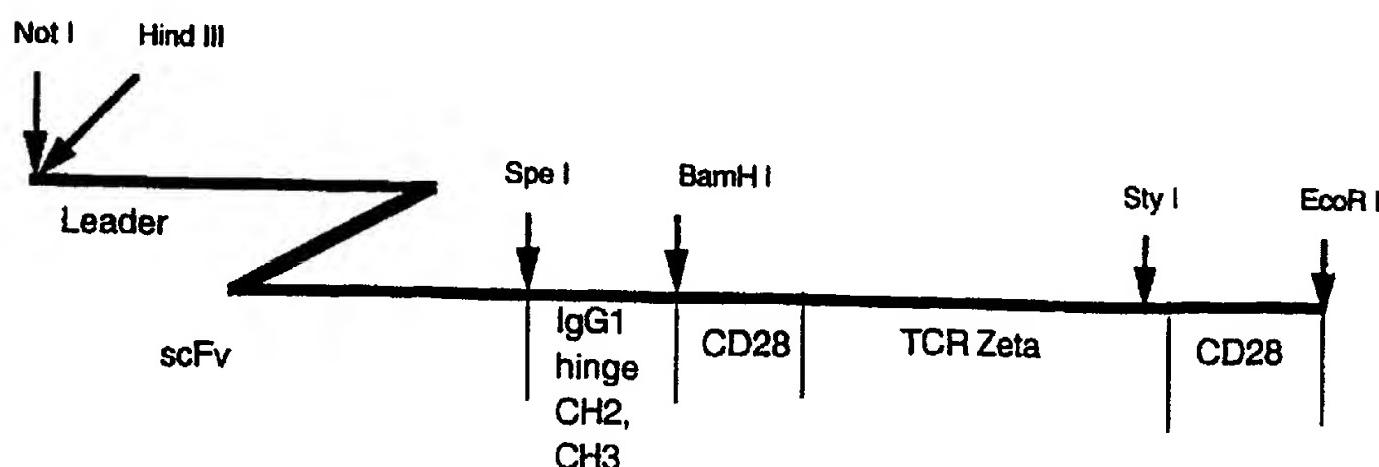
scFv /G1 /CD28 Chimeric Receptor



scFv /G1 /CD28-Zeta fusion Chimeric Receptor



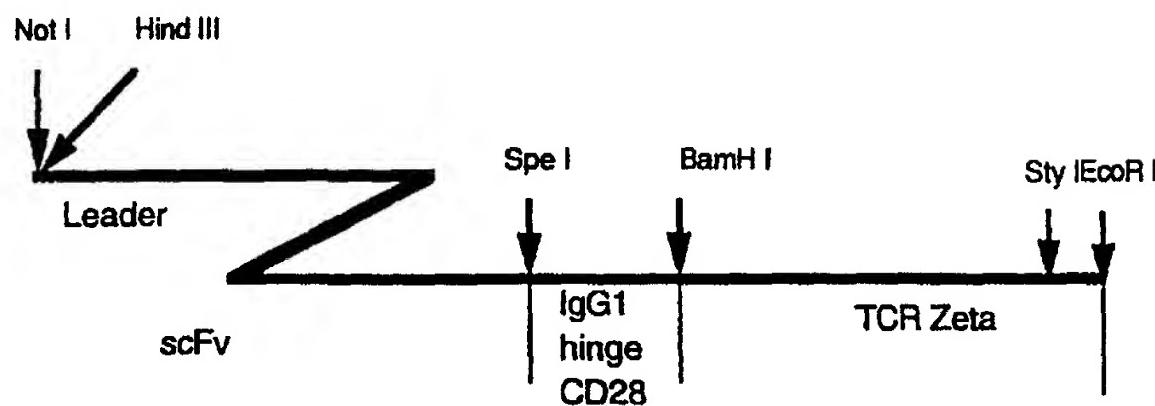
scFv /G1 /CD28-Zeta-CD28 fusion Chimeric Receptor



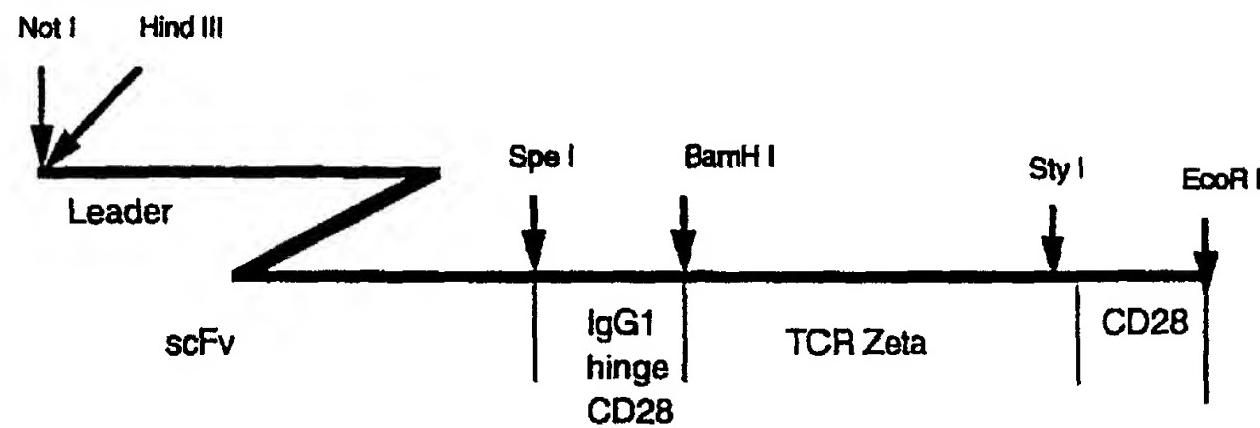
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**FIG. 2c**  
**Construct cassettes cloned into pBluescript SK +**

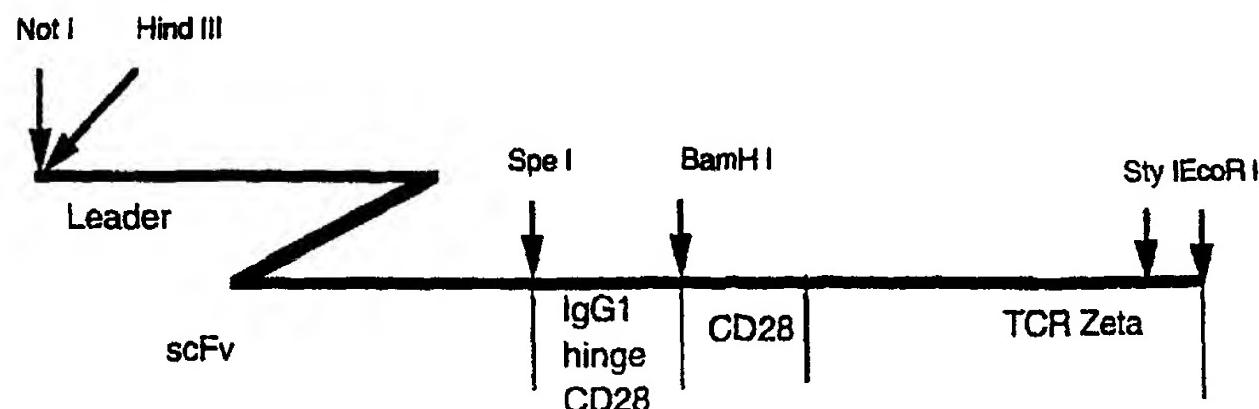
scFv / h.28 / Zeta Chimeric Receptor



scFv / h.28 / Zeta - CD28 fusion Chimeric Receptor



scFv / h.28 / CD28-Zeta fusion Chimeric Receptor



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**FIG. 3**  
**OLIGONUCLEOTIDE SEQUENCES FOR T-BODY CONSTRUCTION**

All oligos listed in the 5' to 3' orientation.

R6490 : ATA TAG CGG CCG CAA GCT TCC ACC ATG TCT GTC CCC ACC CAA  
GTC CTC

R6491 : TGA CCC TCC GCC ACC TGA CCC TCC GCC ACC TGA CCC TCC GCC  
ACC TGA CCC TCC GCC ACC TGA CCC TCC GCC ACC TTT TAC TTC TAC TTT AGT ACC

R6492 : GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA  
GGG TCA GGT GGC GGA GGG TCA GAG GTG CAG CTG GTG CAG TCT

R6493 : TAT ATA CTA GTA GAA GAC ACT GTC ACC AGT

R6516 : TGA CCC TCC GCC ACC TGA CCC TCC GCC ACC TGA CCC TCC GCC  
ACC TGA CCC TCC GCC ACC CGT ACG TTT TAC TTC TAC TTT

R6515 : GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA  
GGG TCA GGT GGC GGA GGG TCA CAG ATT CAG CTG GTG CAG TCT

R6514 : TAT ATA CTA GTC GGG CCC TTC GTT GAG GCA

R6494 : ATA TAA CTA GTA ACT CCA TCA TGT ACT TCA GCC ACT TCG TGC  
CGG TCT TCC TGC CAG CG

R6495 : CGG TGT TGG TGG TCG CGG CGC TGG CGT CGT GGT GGG CTT CGC  
TGG CAG GAA GAC CGG CAC

R6496 : GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC ATC GCG TCG CAG  
CCC CTG TCC CTG CGC CCA

R6497 : TAT ATG GAT CCA GCA GGC CAA AGC TCT GCG CCT CTG GGC GCA  
GGG ACA GGG GCT G

R6506 : TAT ATG GAT CCC GCC TCT GGG CGC AGG GAC AGG GGC TG

R6488 : ATA TAG GAT CCC AAA CTC TGC TAC CTG CTG

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## FIG. 3 (contd.)

R6489 : TAT ATG AAT TCT TAG CGA GGG GGC AGG GCC TGC AT

P3240 : TAT GGA TCC AAG CCC TTT TGG GTG CTG GTG GTG

P3241 : TAT GAA TTC TCA GGA GCG ATA GGC TGC GAA

P3301 : GCC ACC AAG GAC ACC TAC GAC GC

P3302 : CCC CCT CGC AGG AGT AAG AGG AGC AGG CTC CTG CAC AGT GAC  
TAC ATG AAC ATG ACT CCC C

P3303 : CAA GCA TTA CCA GCC CTA TGC CCC ACC ACG CGA CTT CGC AGC  
CTA TCG CTC CTG AGA ATT CAT A

P3304 : TAT GAA TTC TCA GGA GCG ATA G

P3305 : GCA TAG GGCTGG TAA TGC TTG CGG GTG GGC CCG GGG CGG CGG  
GGA GTC ATG TTC ATG TAG T

P3306 : CTC TTA CTC CTG CGA GGG GGC AGG GCC TGC ATG TGA AGG GCG  
TCG TAG GTG TCC TTG GTG GC

S0146 : CGA CTA GTG ACA AAA CTC ACA CAT GCC CAC CGT GCC CAA AAG  
GGA AAC ACC TTT GTC CAA GGT CCC

S0060 : CGA CTA GTG ACA AAA CTC ACA CAT GCC CAC CG

S0061 : TTG GGA TCC AGT TTA CCC GGA GAC AGG GAG AGG CT

T4057 : CTA CTA GTG ACA AAA CTC ACA C

T4058 : TTG GGA TCC AGG GGC TTA GAA GGT CCG GGA AAT AG

T7145 : CTG GAT CCC AAA TTT TGG GTG CTG GTG GTG GTT G

T4060 : GCT CCT GCT GAA CTT CAC TCT GGA GCG ATA GGC TGC GAA GTC G

T4387 : GCG ACT TCG CAG CCT ATC GCT CCA GAG TGA AGT TCA GCA GGA  
GCG

S4700 : TAT GAA TTC TTA GCG AGG GGG CAG GGC CTG CAT G

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## FIG.4

SEQUENCE OF hCTM01 / CD8 / ZETA RECOMBINANT CHIMERIC RECEPTOR

10	20	30	40	
*	*	*	*	
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC M S V P T Q V L G L L L L W>				
50	60	70	80	
*	*	*	*	
CTT ACA GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA GAA TGT CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT L T D A R C D I Q M T Q S P>				
90	100	110	120	
*	*	*	*	
AGT ACT CTC AGT GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT TCA TGA GAG TCA CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA S T L S A S V G D R V T I T>				
130	140	150	160	
*	*	*	*	
TGT AGG AGT AGT AAA AGT CTC CTC CAT AGT AAC GGT GAC ACC ACA TCC TCA TCA TTT TCA GAG GAG GTA TCA TTG CCA CTG TGG C R S S K S L L H S N G D T>				
170	180	190	200	210
*	*	*	*	*
TTC CTC TAT TGG TTC CAG CAG AAA CCA GGT AAA GCC CCA AAG AAG GAG ATA ACC AAG GTC GTC TTT GGT CCA TTT CGG GGT TTC F L Y W F Q Q K P G K A P K>				
220	230	240	250	
*	*	*	*	
CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC AGT GGT GTA CCA GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG TCA CCA CAT GGT L L M Y R M S N L A S G V P>				
260	270	280	290	
*	*	*	*	
TCT AGA TTC AGT GGT AGT GGT AGT GGT ACT GAG TTC ACT CTC AGA TCT AAG TCA CCA TCA CCA TCA TGA CTC AAG TGA GAG S R F S G S G S G T E F T L>				
300	310	320	330	
*	*	*	*	
ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA T I S S L Q P D D F A T Y Y>				
340	350	360	370	
*	*	*	*	
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA C M Q H L E Y P F T F G Q G>				
380	390	400	410	420
*	*	*	*	*
ACT AAA GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT TGA TTT CAT CTT CAT TTT GCA TGC CCA CGG CCT CCC AGT CCA T K V E V K R T G G G G S G>				

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## FIG. 4 (contd.)

430	440	450	460	
*	*	*	*	
GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CCG CCT CCC AGT CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT G G G S G G G S G G G G S>				
470	480	490	500	
*	*	*	*	
GGT GGC GGA GGG TCA CAG ATT CAG CTG GTG CAG TCT GGA GCA CCA CCG CCT CCC AGT GTC TAA GTC GAC CAC GTC AGA CCT CGT G G G S Q I Q L V Q S G A>				
510	520	530	540	
*	*	*	*	
GAG GTG AAG AAG CCT GGA TCT TCT GTG AAG GTG TCT TGT AAG CTC CAC TTC TTC GGA CCT AGA AGA CAC TTC CAC AGA ACA TTC E V K K P G S S V K V S C K>				
550	560	570	580	
*	*	*	*	
GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC ATT AAT TGG ATG CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG TAA TTA ACC TAC A S G Y T F T D Y Y I N W M>				
590	600	610	620	630
*	*	*	*	*
AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA TGG ATT TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT ACC TAA R Q A P G Q G L E W I G W I>				
640	650	660	670	*
*	*	*	*	*
GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC D P G S G N T K Y N E K F K>				
680	690	700	710	*
*	*	*	*	*
GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG G R A T L T V D T S T N T A>				
720	730	740	750	*
*	*	*	*	*
TAC ATG GAG CTG TCT TCT CTG AGA TCT GAG GAC ACA GCA TTC ATG TAC CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG Y M E L S S L R S E D T A F>				
760	770	780	790	*
*	*	*	*	*
TAC TTC TGT GCA AGA GAG AAG ACC ACC TAC TAC TAC GCA ATG ATG AAG ACA CGT TCT CTC TTC TGG TGG ATG ATG ATG CGT TAC Y F C A R E K T T Y Y Y A M>				
800	810	820	830	840
*	*	*	*	*
GAC TAC TGG GGA CAG GGA ACA CTG GTG ACA GTG TCT TCT GCC CTG ATG ACC CCT GTC CCT TGT GAC CAC TGT CAC AGA AGA CGG D Y W G Q G T L V T V S S A>				

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## FIG. 4(contd.)

850	860	870	880	
*	*	*	*	
TCA ACG AAG GGC CCG ACT AGT AAC TCC ATC ATG TAC TTC AGC AGT TGC TTC CCG GGC TGA TCA TTG AGG TAG TAC ATG AAG TCG S T K G P T S N S I M Y F S>				
890	900	910	920	
*	*	*	*	
CAC TTC GTG CCG GTC TTC CTG CCA GCG AAG CCC ACC ACG ACG GTG AAG CAC GGC CAG AAG GAC GGT CGC TTC GGG TGG TGC TGC H F V P V F L P A K P T T T>				
930	940	950	960	
*	*	*	*	
CCA GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC ATC GCG TCG GGT CGC GGC GCT GGT TGT GGC CGC GGG TGG TAG CGC AGC P A P R P P T P A P T I A S>				
970	980	990	1000	
*	*	*	*	
CAG CCC CTG TCC CTG CGC CCA GAG GCG CAG AGC TTT GGC CTG GTC GGG GAC AGG GAC GCG GGT CTC CGC GTC TCG AAA CCG GAC Q P L S L R P E A Q S F G L>				
1010	1020	1030	1040	1050
*	*	*	*	*
CTG GAT CCC AAA CTC TGC TAC CTG CTG GAT GGA ATC CTC TTC GAC CTA GGG TTT GAG AGG ATG GAC GAC CTA CCT TAG GAG AAG L D P K L C Y L L D G I L F>				
1060	1070	1080	1090	
*	*	*	*	
ATC TAT GGT GTC ATT CTC ACT GCC TTG TTC CTG AGA GTG AAG TAG ATA CCA CAG TAA GAG TGA CGG AAC AAG GAC TCT CAC TTC I Y G V I L T A L F L R V K>				
1100	1110	1120	1130	
*	*	*	*	
TTC AGC AGG AGC GCA GAC GCC CCC GCG TAC CAG CAG GCC CAG AAG TCG TCC TCG CGT CTG CGG GGG CGC ATG GTC GTC CGG GTC F S R S A D A P A Y Q Q G Q>				
1140	1150	1160	1170	
*	*	*	*	
AAC CAG CTC TAT AAC GAG CTC AAT CTA GGA CGA AGA GAG GAG TTG GTC GAG ATA TTG CTC GAG TTA GAT CCT GCT TCT CTC CTC N Q L Y N E L N L G R R E E>				
1180	1190	1200	1210	
*	*	*	*	
TAC GAT GTT TTG GAC AAG AGA CGT GGC CGG GAC CCT GAG ATG ATG CTA CAA AAC CTG TTC TCT GCA CGG GCC CTG GGA CTC TAC Y D V L D K R R G R D P E M>				
1220	1230	1240	1250	1260
*	*	*	*	*
GGG GGA AAG CCG AGA AGG AAG AAC CCT CAG GAA GGC CTG TAC CCC CCT TTC GGC TCT TCC TTC TTG GGA GTC CTT CGG GAC ATG G G K P R R K N P Q E G L Y>				

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## FIG.4 (contd)

1270            1280            1290            1300

AAT GAA CTG CAG AAA GAT AAG ATG GCG GAG GCC TAC AGT GAG  
 TTA CTT GAC GTC TTT CTA TTC TAC CGC CTC CGG ATG TCA CTC  
 N E L Q K D K M A E A Y S E>

1310            1320            1330            1340

ATT GGG ATG AAA GGC GAG CGC CGG AGG GGC AAG GGG CAC GAT  
 TAA CCC TAC TTT CCG CTC CGG GCC TCC CCG TTC CCC GTG CTA  
 I G M K G E R R R G K G H D>

1350            1360            1370            1380

GCG CTT TAC CAG GGT CTC AGT ACA GCC ACC AAG GAC ACC TAC  
 CCG GAA ATG GTC CCA GAG TCA TGT CGG TGG TTC CTG TGG ATG  
 G L Y Q G L S T A T K D T Y>

1390            1400            1410            1420

GAC GCC CTT CAC ATG CAG GCC CTG CCC CCT CGC TAA  
 CTG CGG GAA GTG TAC GTC CGG GAC GGG GGA GCG ATT  
 D A L H M Q A L P P R \*

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## FIG.5

SEQUENCE OF hCTM01 / CD8 /Zeta-CD28 FUSION RECOMBINANT CHIMERIC RECEPTOR

10	20	30	40	
*	*	*	*	
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG CTT ACA TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC GAA TGT m s v p t q v l g 1 1 1 1 1 w 1 t>				
50	60	70	80	90
*	*	*	*	*
GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA AGT ACT CTC AGT CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT TCA TGA GAG TCA d a r c D I Q M T Q S P S T L S>				
100	110	120	130	140
*	*	*	*	*
GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT TGT AGG AGT AGT AAA AGT CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA ACA TCC TCA TCA TTT TCA A S V G D R V T I T C R S S K S>				
150	160	170	180	190
*	*	*	*	*
CTC CTC CAT AGT AAC GGT GAC ACC TTC CTC TAT TGG TTC CAG CAG AAA GAG GAG GTA TCA TTG CCA CTG TGG AAG GAG ATA ACC AAG GTC GTC TTT L L H S N G D T F L Y W F Q Q K>				
200	210	220	230	240
*	*	*	*	*
CCA GGT AAA GCC CCA AAG CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC GGT CCA TTT CGG GGT TTC GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG P G K A P K L L M Y R M S N L A>				
250	260	270	280	
*	*	*	*	
AGT GGT GTA CCA TCT AGA TTC AGT GGT AGT GGT AGT GGT ACT GAG TTC TCA CCA CAT GGT AGA TCT AAG TCA CCA TCA CCA TCA TGA CTC AAG S G V P S R F S G S G S G T E F>				
290	300	310	320	330
*	*	*	*	*
ACT CTC ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT TGA GAG TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA T L T I S S L Q P D D F A T Y Y>				
340	350	360	370	380
*	*	*	*	*
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT ACT AAA ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA TGA TTT C M Q H L E Y P F T F G Q G T K>				
390	400	410	420	430
*	*	*	*	*
GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAT CTT CAT TTT GCA TGC CCA CGG CCT CCC AGT CCA CGG CCT CCC AGT V E V K R T G G G G S G G G G G S>				

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## FIG. 5 (contd.)

440	450	460	470	480
*	*	*	*	*
GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAG CCA CGG CCT CCC AGT CCA CGG CCT CCC AGT CCA CGG CCT CCC AGT GTC G G G G S G G G S G G G G G S Q>				
490	500	510	520	
*	*	*	*	
ATT CAG CTG GTG CAG TCT GGA GCA GAG GTG AAG AAG CCT GGA TCT TCT TAA GTC GAC CAC GTC AGA CCT CGT CTC CAC TTC TTC GGA CCT AGA AGA I Q L V Q S G A E V K K P G S S>				
530	540	550	560	570
*	*	*	*	*
GTG AAG GTG TCT TGT AAG GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC CAC TTC CAC AGA ACA TTC CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG V K V S C K A S G Y T F T D Y Y>				
580	590	600	610	620
*	*	*	*	*
ATT AAT TGG ATG AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA TAA TTA ACC TAC TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT I N W M R Q A P G Q G L E W I G>				
630	640	650	660	670
*	*	*	*	*
TGG ATT GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG ACC TAA CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC W I D P G S G N T K Y N E K F K>				
680	690	700	710	720
*	*	*	*	*
GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC TAC ATG CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG ATG TAC G R A T L T V D T S T N T A Y M>				
730	740	750	760	
*	*	*	*	
GAG CTG TCT CTG AGA TCT GAG GAC ACA GCA TTC TAC TTC TGT GCA CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG ATG AAG ACA CGT E L S S L R S E D T A F Y F C A>				
770	780	790	800	810
*	*	*	*	*
AGA GAG AAG ACC ACC TAC TAC GCA ATG GAC TAC TGG GGA CAG GGA TCT CTC TTC TGG TGG ATG ATG CGT TAC CTG ATG ACC CCT GTC CCT R E K T T Y Y A M D Y W G Q G>				
820	830	840	850	860
*	*	*	*	*
ACA CTG GTG ACA GTG TCT TCT GCC TCA ACG AAG GGC CCG ACT AGT AAC TGT GAC CAC TGT CAC AGA AGA CGG AGT TGC TTC CCG GGC TGA TCA TTG T L V T V S S A S T K G P T S N>				
870	880	890	900	910
*	*	*	*	*
TCC ATC ATG TAC TTC AGC CAC TTC GTG CGG GTC TTC CTG CCA GCG AAG AGG TAG TAC ATG AAG TCG GTG AAG CAC CGC CAG AAG GAC GGT CGC TTC S I M Y F S H F V P V F L P A K>				

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## FIG. 5(contd.)

920	930	940	950	960
*	*	*	*	*
CCC ACC ACG ACG CCA GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC ATC GGG TGG TGC TGC GGT CGC GGC GCT GGT GGT TGT GGC CGC GGG TGG TAG P T T T P A P R P P T P A P T I>				
970	980	990	1000	
*	*	*	*	
GCG TCG CAG CCC CTG TCC CTG CGC CCA GAG GCG CAG ACC TTT GGC CTG CGC AGC GTC GGG GAC AGG GAC GCG GGT CTC CGC GTC TCG AAA CCG GAC A S Q P L S L R P E A Q S F G L>				
1010	1020	1030	1040	1050
*	*	*	*	*
CTG GAT CCC AAA CTC TGC TAC CTG CTG GAT GGA ATC CTC TTC ATC TAT GAC CTA GGG TTT GAG ACG ATG GAC GAC CTA CCT TAG GAG AAG TAG ATA L D P K L C Y L L D G I L F I Y>				
1060	1070	1080	1090	1100
*	*	*	*	*
GGT GTC ATT CTC ACT GCC TTG TTC CTG AGA GTG AAG TTC AGC AGG AGC CCA CAG TAA GAG TGA CGG AAC AAG GAC TCT CAC TTC AAG TCG TCC TCG G V I L T A L F L R V K F S R S>				
1110	1120	1130	1140	1150
*	*	*	*	*
GCA GAC GCC CCC GCG TAC CAG CAG GGC CAG AAC CAG CTC TAT AAC GAG CGT CTG CGG GGG CGC ATG GTC GTC CGG GTC TTG GTC GAG ATA TTG CTC A D A P A Y Q Q G Q N Q L Y N E>				
1160	1170	1180	1190	1200
*	*	*	*	*
CTC AAT CTA GGA CGA AGA GAG GAG TAC GAT GTT TTG GAC AAG AGA CGT GAG TTA GAT CCT GCT TCT CTC CTC ATG CTA CAA AAC CTG TTC TCT GCA L N L G R R E E Y D V L D K R R>				
1210	1220	1230	1240	
*	*	*	*	
GGC CGG GAC CCT GAG ATG GGG GGA AAG CCG AGA AGG AAG AAC CCT CAG CCG GCC CTG GGA CTC TAC CCC CCT TTC GGC TCT TCC TTC TTG GGA GTC G R D P E M G G K P R R K N P Q>				
1250	1260	1270	1280	1290
*	*	*	*	*
GAA GGC CTG TAC AAT GAA CTG CAG AAA GAT AAG ATG GCG GAG GCC TAC CTT CCG GAC ATG TTA CTT GAC GTC TTT CTA TTC TAC CGC CTC CGG ATG E G L Y N E L Q K D K M A E A Y>				
1300	1310	1320	1330	1340
*	*	*	*	*
AGT GAG ATT GGG ATG AAA GGC GAG CGC CGG AGG GGC AAG GGG CAC GAT TCA CTC TAA CCC TAC TTT CCG CTC CGG GGC TCC CGG TTC CCC GTG CTA S E I G M K G E R R R G K G H D>				
1350	1360	1370	1380	1390
*	*	*	*	*
GGC CTT TAC CAG GGT CTC AGT ACA GCC ACC AAG GAC ACC TAC GAC GCC CCG GAA ATG GTC CCA GAG TCA TGT CGG TGG TTC CTG TGG ATG CTG CGG G L Y Q G L S T A T K D T Y D A>				

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## FIG.5(contd.)

1400	1410	1420	1430	1440
*	*	*	*	*
CTT CAC ATG CAG GCC CTG CCC CCT CGC AGG AGT AAG AGG AGC AGG CTC				
GAA GTG TAC GTC CGG GAC GGG GGA GCG TCC TCA TTC TCC TCG TCC GAG				
L H M Q A L P P R R S K R S R L>				
1450                  1460                  1470                  1480				
*	*	*	*	
CTG CAC AGT GAC TAC ATG AAC ATG ACT CCC CGC CGC CCC GGG CCC ACC				
GAC GTG TCA CTG ATG TAC TTG TAC TGA GGG GCG GCG GGG CCC GGG TGG				
L H S D Y M N M T P R R P G P T>				
1490                  1500                  1510                  1520                  1530				
*	*	*	*	*
CGC AAG CAT TAC CAG CCC TAT GCC CCA CCA CGC GAC TTC GCA GCC TAT				
GCG TTC GTA ATG GTC GGG ATA CGG CGT GGT GCG CTG AAG CGT CGG ATA				
R K H Y Q P Y A P P R D F A A Y>				
1540				
*				
CGC TCC TGA				
GCG AGG ACT				
R S *				

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**FIG.6**  
**SEQUENCE OF hCTM01 /CD8 / CD28 RECOMBINANT CHIMERIC  
 RECEPTOR**

10	20	30	40										
*	*	*	*										
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG													
TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC													
M	S	V	P	T	Q	V	L	G	L	L	L	W>	
50	60	70	80										
*	*	*	*										
CTT ACA GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA													
GAA TGT CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT													
L	T	D	A	R	C	D	I	Q	M	T	Q	S	P>
90	100	110	120										
*	*	*	*										
AGT ACT CTC AGT GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT													
TCA TGA GAG TCA CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA													
S	T	L	S	A	S	V	G	D	R	V	T	I	T>
130	140	150	160										
*	*	*	*										
TGT AGG AGT AGT AAA AGT CTC CTC CAT AGT AAC GGT GAC ACC													
ACA TCC TCA TCA TTT TCA GAG GAG GTA TCA TTG CCA CTG TGG													
C	R	S	S	K	S	L	L	H	S	N	G	D	T>
170	180	190	200	210									
*	*	*	*	*									
TTC CTC TAT TGG TTC CAG CAG AAA CCA GGT AAA GCC CCA AAG													
AAG GAG ATA ACC AAG GTC GTC TTT GGT CCA TTT CGG GGT TTC													
F	L	Y	W	F	Q	Q	K	P	G	K	A	P	K>
220	230	240	250										
*	*	*	*										
CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC AGT GGT GTA CCA													
GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG TCA CCA CAT GGT													
L	L	M	Y	R	M	S	N	L	A	S	G	V	P>
260	270	280	290										
*	*	*	*										
TCT AGA TTC AGT GGT AGT GGT ACT GAG TTC ACT CTC													
AGA TCT AAG TCA CCA TCA CCA CCA TGA CTC AAG TGA GAG													
S	R	F	S	G	S	G	S	G	T	E	F	T	L>
300	310	320	330										
*	*	*	*										
ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT													
TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA													
T	I	S	S	L	Q	P	D	D	F	A	T	Y	Y>
340	350	360	370										
*	*	*	*										
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT													
ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA													
C	M	Q	H	L	E	Y	P	F	T	F	G	Q	G>

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## FIG. 6 (contd.)

380	390	400	410	420
*	*	*	*	*
ACT AAA GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT TGA TTT CAT CTT CAT TTT GCA TGC CCA CCG CCT CCC AGT CCA T K V E V K R T G G G G S G>				
430	440	450	460	
*	*	*	*	
GCC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CCG CCT CCC AGT CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT G G G S G G G S G G G G S G>				
470	480	490	500	
*	*	*	*	
GGT GGC GGA GGG TCA CAG ATT CAG CTG GTG CAG TCT GGA GCA CCA CGG CCT CCC AGT GTC TAA GTC GAC CAC GTC AGA CCT CGT G G G S Q I Q L V Q S G A>				
510	520	530	540	
*	*	*	*	
GAG GTG AAG AAG CCT GGA TCT TCT GTG AAG GTG TCT TGT AAG CTC CAC TTC TTC GGA CCT AGA AGA CAC TTC CAC AGA ACA TTC E V K K P G S S V K V S C K>				
550	560	570	580	
*	*	*	*	
GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC ATT AAT TGG ATG CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG TAA TTA ACC TAC A S G Y T F T D Y Y I N W M>				
590	600	610	620	630
*	*	*	*	*
AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA TGG ATT TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT ACC TAA R Q A P G Q G L E W I G W I>				
640	650	660	670	
*	*	*	*	
GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC D P G S G N T K Y N E K F K>				
680	690	700	710	
*	*	*	*	
GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG G R A T L T V D T S T N T A>				
720	730	740	750	
*	*	*	*	
TAC ATG GAG CTG TCT CTG AGA TCT GAG GAC ACA GCA TTC ATG TAC CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG Y M E L S S L R S E D T A F>				
760	770	780	790	
*	*	*	*	
TAC TTC TGT GCA AGA GAG AAG ACC ACC TAC TAC TAC GCA ATG ATG AAG ACA CGT TCT CTC TTC TGG TGG ATG ATG ATG CGT TAC Y F C A R E K T T Y Y Y Y A M>				

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## FIG. 6 (contd.)

800	810	820	830	840
*	*	*	*	*
GAC TAC TGG GGA CAG GGA ACA CTG GTG ACA GTG TCT TCT GCC CTG ATG ACC CCT GTC CCT TGT GAC CAC TGT CAC AGA AGA CGG D Y W G Q G T L V T V S S A>				
850	860	870	880	*
*	*	*	*	*
TCA ACG AAG GGC CCG ACT AGT AAC TCC ATC ATG TAC TTC AGC AGT TGC TTC CCG GGC TGA TCA TTG AGG TAG TAC ATG AAG TCG S T K G P T S N S I M Y F S>				
890	900	910	920	*
*	*	*	*	*
CAC TTC GTG CCG GTC TTC CTG CCA GCG AAG CCC ACC ACG ACG GTG AAG CAC GGC CAG AAG GAC GGT CGC TTC GGG TGG TGC TGC H F V P V F L P A K P T T T				
930	940	950	960	*
*	*	*	*	*
CCA GCG CCG CGA CCA CCA ACA CCG GCG CCC ACC ATC GCG TCG GGT CGC GGC CCT GGT GGT TGT GGC CGC GGG TGG TAG CGC AGC P A P R P P T P A P T I A S>				
970	980	990	1000	*
*	*	*	*	*
CAG CCC CTG TCC CTG CGC CCA GAG GCG GGA TCC AAG CCC TTT GTC GGG GAC AGG GAC GCG GGT CTC CGC CCT AGG TTC GGG AAA Q P L S L /R P E A G S K P F>				
1010	1020	1030	1040	1050
*	*	*	*	*
TGG GTG CTG GTG GTG GTT GGT GGA GTC CTG GCT TGC TAT AGC ACC CAC GAC CAC CAA CCA CCT CAG GAC CGA ACG ATA TCG W V L V V V G G V L A C Y S>				
1060	1070	1080	1090	*
*	*	*	*	*
TTG CTA GTA ACA GTG GCC TTT ATT ATT TTC TGG GTG AGG AGT AAC GAT CAT TGT CAC CGG AAA TAA TAA AAG ACC CAC TCC TCA L L V T V A F I I F W V R S>				
1100	1110	1120	1130	*
*	*	*	*	*
AAG AGG AGC AGG CTC CTG CAC AGT GAC TAC ATG AAC ATG ACT TTC TCC TCG TCC GAG GAC GTG TCA CTG ATG TAC TTG TAC TGA K R S R L L H S D Y M N M T>				
1140	1150	1160	1170	*
*	*	*	*	*
CCC CGC CGC CCC GGG CCC ACC CGC AAG CAT TAC CAG CCC TAT GGG GCG GCG GGG CCC GGG TGG GCG TTC GTA ATG GTC GGG ATA P R R P G P T R K H Y Q P Y>				
1180	1190	1200	1210	*
*	*	*	*	*
GCC CCA CCA CGC GAC TTC GCA GCC TAT CGC TCC TGA CGG GGT GGT GCG CTG AAG CGT CGG ATA GCG AGG ACT A P P R D F A A Y R S *				

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**FIG. 7**  
**SEQUENCE OF hCTM01 / G1 / ZETA RECOMBINANT CHIMERIC**  
**RECEPTOR**

10	20	30	40	
*	*	*	*	
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG CTT ACA TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC GAA TGT M S V P T Q V L G L L L W L T>				
50	60	70	80	90
*	*	*	*	*
GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA AGT ACT CTC AGT CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT TCA TGA GAG TCA D A R C D I Q M T Q S P S T L S>				
100	110	120	130	140
*	*	*	*	*
GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT TGT AGG AGT AGT AAA AGT CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA ACA TCC TCA TCA TTT TCA A S V G D R V T I T C R S S K S>				
150	160	170	180	190
*	*	*	*	*
CTC CTC CAT AGT AAC GGT GAC ACC TTC CTC TAT TGG TTC CAG CAG AAA GAG GAG GTA TCA TTG CCA CTG TGG AAG GAG ATA ACC AAG GTC GTC TTT L L H S N G D T F L Y W F Q Q K>				
200	210	220	230	240
*	*	*	*	*
CCA GGT AAA GCC CCA AAG CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC GGT CCA TTT CGG GGT TTC GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG P G K A P K L L M Y R M S N L A>				
250	260	270	280	
*	*	*	*	
AGT GGT GTA CCA TCT AGA TTC AGT GGT AGT GGT ACT GAG TTC TCA CCA CAT GGT AGA TCT AAG TCA CCA TCA CCA TCA CCA TGA CTC AAG S G V P S R F S G S G S G T E F>				
290	300	310	320	330
*	*	*	*	*
ACT CTC ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT TGA GAG TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA T L T I S S L Q P D D F A T Y Y>				
340	350	360	370	380
*	*	*	*	*
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT ACT AAA ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA TGA TTT C M Q H L E Y P F T F G Q G T K>				
390	400	410	420	430
*	*	*	*	*
GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT GCC GGA GGG TCA CAT CTT CAT TTT GCA TGC CCA CGG CCT CCC AGT CCA CGG CCT CCC AGT V E V X R T G G G G S G G G G G S>				

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## FIG. 7 (contd.)

440 \* 450 \* 460 \* 470 \* 480 \*  
 GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAG  
 CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT GTC  
 G G G G S G G G S G G G G S Q>  
  
 490 \* 500 \* 510 \* 520 \*  
 ATT CAG CTG GTG CAG TCT GGA GCA GAG GTG AAG AAG CCT GGA TCT TCT  
 TAA GTC GAC CAC GTC AGA CCT CGT CTC CAC TTC TTC GGA CCT AGA AGA  
 I Q L V Q S G A E V K K P G S S>  
  
 530 \* 540 \* 550 \* 560 \* 570 \*  
 GTG AAG GTG TCT TGT AAG GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC  
 CAC TTC CAC AGA ACA TTC CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG  
 V K V S C K A S G Y T F T D Y Y>  
  
 580 \* 590 \* 600 \* 610 \* 620 \*  
 ATT AAT TGG ATG AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA  
 TAA TTA ACC TAC TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT  
 I N W M R Q A P G Q G L E W I G>  
  
 630 \* 640 \* 650 \* 660 \* 670 \*  
 TGG ATT GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG  
 ACC TAA CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC  
 W I D P G S G N T K Y N E K F K>  
  
 680 \* 690 \* 700 \* 710 \* 720 \*  
 GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC TAC ATG  
 CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG ATG TAC  
 G R A T L T V D T S T N T A Y M>  
  
 730 \* 740 \* 750 \* 760 \*  
 GAG CTG TCT CTG AGA TCT GAG GAC ACA GCA TTC TAC TTC TGT GCA  
 CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG ATG AAG ACA CGT  
 E L S S L R S E D T A F Y F C A>  
  
 770 \* 780 \* 790 \* 800 \* 810 \*  
 AGA GAG AAG ACC ACC TAC TAC GCA ATG GAC TAC TGG GGA CAG GGA  
 TCT CTC TTC TGG TGG ATG ATG CGT TAC CTG ATG ACC CCT GTC CCT  
 R E K T T Y Y A M D Y W G Q G>  
  
 820 \* 830 \* 840 \* 850 \* 860 \*  
 ACA CTG GTG ACA GTG TCT TCT GCC TCA ACG AAG GGC CCG ACT AGT GAC  
 TGT GAC CAC TGT CAC AGA AGA CGG AGT TGC TTC CCG GGC TGA TCA CTG  
 T L V T V S S A S T K G P T S D>  
  
 870 \* 880 \* 890 \* 900 \* 910 \*  
 AAA ACT CAC ACA TGC CCA CGG TGC CCA GCA CCT GAA CTC CTG GGG GGA  
 TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT  
 K T H T C P P C P A P E L L G G>

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## FIG. 7 (contd.)

920	930	940	950	960
*	*	*	*	*
CCG TCA GTC TTC CTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG P S V F L F P P K P K D T L M I>				
970	980	990	1000	
*	*	*	*	
TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC GTG AGC CAC GAA AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CTG CAC TCG GTG CTT S R T P E V T C V V V D V S H E>				
1010	1020	1030	1040	1050
*	*	*	*	*
GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA D P E V K F N W Y V D G V E V H>				
1060	1070	1080	1090	1100
*	*	*	*	*
AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA N A K T K P R E E Q Y N S T Y R>				
1110	1120	1130	1140	1150
*	*	*	*	*
GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC V V S V L T V L H Q D W L N G X>				
1160	1170	1180	1190	1200
*	*	*	*	*
GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC E Y K C K V S N K A L P A P I E>				
1210	1220	1230	1240	
*	*	*	*	
AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG K T I S K A K G Q P R E P Q V Y>				
1250	1260	1270	1280	1290
*	*	*	*	*
ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAC CAG GTC AGC CTG TGG GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC T L P P S R E E M T K N Q V S L>				
1300	1310	1320	1330	1340
*	*	*	*	*
ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG TGG AGC GAC CAG TTT CGG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC T C L V K G F Y P S D I A V E W>				
1350	1360	1370	1380	1390
*	*	*	*	*
GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC E S N G Q P E N N Y K T T P P V>				
1400	1410	1420	1430	1440
*	*	*	*	*
CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTC L D S D G S F F L Y S K L T V D>				

## 21/40 FIG. 7 (contd.)

1450	1460	1470	1480	
*	*	*	*	
AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACC AGG AGG CAC TAC GTA K S R W Q Q G N V F S C S V M H>				
1490	1500	1510	1520	1530
*	*	*	*	*
GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC E A L H N H Y T Q K S L S L S P>				
1540	1550	1560	1570	1580
*	*	*	*	*
GGT AAA CTG GAT CCC AAA CTC TGC TAC CTG CTG GAT GGA ATC CTC TTC CCA TTT GAC CTA GGG TTT GAG ACG ATG GAC GAC CTA CCT TAG GAG AAG G K L D P K L C Y L L D G I L F>				
1590	1600	1610	1620	1630
*	*	*	*	*
ATC TAT GGT GTC ATT CTC ACT GCC TTG TTC CTG AGA GTG AAG TTC AGC TAG ATA CCA CAG TAA GAG TGA CGG AAC AAG GAC TCT CAC TTC AAG TCG I Y G V I L T A L F L R V K F S>				
1640	1650	1660	1670	1680
*	*	*	*	*
AGG AGC GCA GAC GCC CCC GCG TAC CAG CAG GGC CAG AAC CAG CTC TAT TCC TCG CGT CTG CGG GGG CGC ATG GTC GTC CGG GTC TTG GTC GAG ATA R S A D A P A Y Q Q G Q N Q L Y>				
1690	1700	1710	1720	
*	*	*	*	
AAC GAG CTC AAT CTA GGA CGA AGA GAG GAG TAC GAT GAT GTT TTG GAC AAG TTG CTC GAG TTA GAT CCT GCT TCT CTC CTC ATG CTA CAA AAC CTG TTC N E L N L G R R E E Y D V L D K>				
1730	1740	1750	1760	1770
*	*	*	*	*
AGA CGT GGC CGG GAC CCT GAG ATG GGG GGA AAG CCG AGA AGG AAG AAC TCT GCA CCG GCC CTG GGA CTC TAC CCC CCT TTC GGC TCT TCC TTC TTG R R G R D P E M G G K P R R K N>				
1780	1790	1800	1810	1820
*	*	*	*	*
CCT CAG GAA GGC CTG TAC AAT GAA CTG CAG AAA GAT AAG ATG GCG GAG GGA GTC CTT CGG GAC ATG TTA CTT GAC GTC TTT CTA TTC TAC CGC CTC P Q E G L Y N E L Q K D K M A E>				
1830	1840	1850	1860	1870
*	*	*	*	*
GCC TAC AGT GAG ATT GGG ATG AAA GGC GAG CGC CGG AGG GGC AAG GGG CGG ATG TCA CTC TAA CCC TAC TTT CCG CTC GCG GCC TCC CCG TTC CCC A Y S E I G M K G E R R R G K G>				
1880	1890	1900	1910	1920
*	*	*	*	*
CAC GAT GGC CTT TAC CAG GGT CTC AGT ACA GCC ACC AAG GAC ACC TAC GTG CTA CCG GAA ATG GTC CCA GAG TCA TGT CGG TGG TTC CTG TGG ATG H D G L Y Q G L S T A T K D T Y>				
1930	1940	1950		
*	*	*		
GAC GCC CTT CAC ATG CAG GCC CTG CCC CCT CGC TAA CTG CGG GAA GTG TAC GTC CGG GAC GGG GGA GCG ATT D A L H M Q A L P P R *				

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**FIG. 8**  
**SEQUENCE OF hCTMO1/G1/ZETA-CD28 FUSION RECOMBINANT  
 CHIMERIC RECEPTOR**

10	20	30	40	
*	*	*	*	
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTC CTG CTG CTG CTG TGG CTT ACA				
TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC GAA TGT				
M	S	V	P	T
Q	V	L	G	L
L	L	L	L	W
L	L	W	L	T>
50	60	70	80	90
*	*	*	*	*
GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA AGT ACT CTC AGT				
CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT TCA TGA GAG TCA				
D	A	R	C	D
I	Q	M	T	Q
S	P	S	T	L
P	S	T	L	S>
100	110	120	130	140
*	*	*	*	*
GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT TGT AGG AGT AGT AAA AGT				
CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA ACA TCC TCA TCA TTT TCA				
A	S	V	G	D
R	V	T	I	T
C	T	C	R	S
S	S	S	K	S>
150	160	170	180	190
*	*	*	*	*
CTC CTC CAT AGT AAC GGT GAC ACC TTC CTC TAT TGG TTC CAG CAG AAA				
GAG GAG GTA TCA TTG CCA CTG TGG AAG GAG ATA ACC AAG GTC GTC TTT				
L	L	H	S	N
S	N	G	D	T
F	L	Y	W	F
Q	Q	Q	Q	K>
200	210	220	230	240
*	*	*	*	*
CCA GGT AAA GCC CCA AAG CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC				
GGT CCA TTT CGG GGT TTC GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG				
P	G	K	A	P
K	L	L	M	Y
R	M	S	N	L
M	S	N	L	A>
250	260	270	280	
*	*	*	*	
AGT GGT GTA CCA TCT AGA TTC AGT GGT AGT GGT ACT GAG TTC				
TCA CCA CAT GGT AGA TCT AAG TCA CCA TCA CCA TCA CCA TGA CTC AAG				
S	G	V	P	S
R	F	S	R	F
G	S	G	S	G
S	G	T	E	F>
290	300	310	320	330
*	*	*	*	*
ACT CTC ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT				
TGA GAG TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA				
T	L	T	I	S
L	S	S	S	L
Q	P	D	D	F
P	D	D	F	A
F	A	T	Y	Y>
340	350	360	370	380
*	*	*	*	*
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT ACT AAA				
ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA TGA TTT				
C	M	Q	H	L
M	Q	H	L	E
Q	H	L	E	V
H	L	E	V	P
L	E	V	P	F
E	V	K	R	T
V	E	V	K	R
K	R	T	G	G
T	G	G	G	G
G	G	G	G	G
G	G	G	G	S>

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## FIG. 8 (contd.)

440            450            460            470            480  
 \*            \*            \*            \*            \*  
 GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAG  
 CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT CCA CCG CCT CCC AGT GTC  
 G G G G S G G G S G G G G G S Q>  
  
 490            500            510            520  
 \*            \*            \*            \*  
 ATT CAG CTG GTG CAG TCT GGA GCA GAG GTG AAG AAG CCT GGA TCT TCT  
 TAA GTC GAC CAC GTC AGA CCT CGT CTC CAC TTC TTC GGA CCT AGA AGA  
 I Q L V Q S G A E V K K P G S S>  
  
 530            540            550            560            570  
 \*            \*            \*            \*            \*  
 GTG AAG GTG TCT TGT AAG GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC  
 CAC TTC CAC AGA ACA TTC CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG  
 V K V S C K A S G Y T F T D Y Y>  
  
 580            590            600            610            620  
 \*            \*            \*            \*            \*  
 ATT AAT TGG ATG AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA  
 TAA TTA ACC TAC TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT  
 I N W M R Q A P G Q G L E W I G>  
  
 630            640            650            660            670  
 \*            \*            \*            \*            \*  
 TGG ATT GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG  
 ACC TAA CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC  
 W I D P G S G N T K Y N E K F K>  
  
 680            690            700            710            720  
 \*            \*            \*            \*            \*  
 GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC TAC ATG  
 CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG ATG TAC  
 G R A T L T V D T S T N T A Y M>  
  
 730            740            750            760  
 \*            \*            \*            \*  
 GAG CTG TCT TGT AGA TCT GAG GAC ACA GCA TTC TAC TTC TGT GCA  
 CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG ATG AAG ACA CGT  
 E L S S L R S E D T A F Y F C A>  
  
 770            780            790            800            810  
 \*            \*            \*            \*            \*  
 AGA GAG AAG ACC ACC TAC TAC GCA ATG GAC TAC TGG GGA CAG GGA  
 TCT CTC TTC TGG TGG ATG ATG CGT TAC CTG ATG ACC CCT GTC CCT  
 R E K T T Y Y A M D Y W G Q G>  
  
 820            830            840            850            860  
 \*            \*            \*            \*            \*  
 ACA CTG GTG ACA GTG TCT TGT GCC TCA ACG AAG GGC CCG ACT AGT GAC  
 TGT GAC CAC TGT CAC AGA AGA CGG AGT TGC TTC CCG GGC TGA TCA CTG  
 T L V T V S S A S T K G P T S D>  
  
 870            880            890            900            910  
 \*            \*            \*            \*            \*  
 AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA  
 TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT  
 K T H T C P P C P A P E L L G G>

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## FIG. 8(contd.)

920	930	940	950	960
*	*	*	*	*
CCG TCA GTC TTC CTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG P S V F L F P P K P K D T L M I>				
970	980	990	1000	*
*	*	*	*	*
TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT S R T P E V T C V V V D V S H E>				
1010	1020	1030	1040	1050
*	*	*	*	*
GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA D P E V K F N W Y V D G V E V H>				
1060	1070	1080	1090	1100
*	*	*	*	*
AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA N A K T K P R E E Q Y N S T Y R>				
1110	1120	1130	1140	1150
*	*	*	*	*
GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC V V S V L T V L H Q D W L N G K>				
1160	1170	1180	1190	1200
*	*	*	*	*
GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC E Y K C K V S N K A L P A P I E>				
1210	1220	1230	1240	*
*	*	*	*	*
AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG K T I S K A K G Q P R E P Q V Y>				
1250	1260	1270	1280	1290
*	*	*	*	*
ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAC AAC CAG GTC AGC CTG TGG GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC T L P P S R E E M T K N Q V S L>				
1300	1310	1320	1330	1340
*	*	*	*	*
ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG TGG AGC GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC T C L V K G F Y P S D I A V E W>				
1350	1360	1370	1380	1390
*	*	*	*	*
GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC E S N G Q P E N N Y K T T P P V>				
1400	1410	1420	1430	1440
*	*	*	*	*
CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTC L D S D G S F F L Y S K L T V D>				

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FIG. 8 (contd.)

1450	1460	1470	1480
*	*	*	*
AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT			
TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA			
K S R W Q Q G N V F S C S V M H>			
1490	1500	1510	1520
*	*	*	*
GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG			
CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC			
E A L H N H Y T Q K S L S L S P>			
1540	1550	1560	1570
*	*	*	*
GGT AAA CTG GAT CCC AAA CTC TGC TAC CTG CTG GAT GGA ATC CTC TTC			
CCA TTT GAC CTA GGG TTT GAG ACG ATG GAC GAC CTA CCT TAG GAG AAG			
G K L D P K L C Y L L D G I L F>			
1590	1600	1610	1620
*	*	*	*
ATC TAT GGT GTC ATT CTC ACT GCC TTG TTC CTG AGA GTG AAG TTC AGC			
TAG ATA CCA CAG TAA GAG TGA CGG AAC AAG GAC TCT CAC TTC AAG TCG			
I Y G V I L T A L F L R V K F S>			
1640	1650	1660	1670
*	*	*	*
AGG AGC GCA GAC GCC CCC GCG TAC CAG CAG GGC CAG AAC CAG CTC TAT			
TCC TCG CGT CTG CGG GGG CGC ATG GTC GTC CCG GTC TTG GTC GAG ATA			
R S A D A P A Y Q Q G Q N Q L Y>			
1690	1700	1710	1720
*	*	*	*
AAC GAG CTC AAT CTA GGA CGA AGA GAG GAG TAC GAT GTT TTG GAC AAG			
TTG CTC GAG TTA GAT CCT CCT CTC CTC ATG CTA CAA AAC CTG TTC			
N E L N L G R R E E Y D V L D K>			
1730	1740	1750	1760
*	*	*	*
AGA CGT GGC CGG GAC CCT GAG ATG GGG GGA AAG CCG AGA AGG AAG AAC			
TCT GCA CGG GCC CTG GGA CTC TAC CCC CCT TTC GGC TCT TCC TTC TTG			
R R G R D P E M G G K P R R K N>			
1780	1790	1800	1810
*	*	*	*
CCT CAG GAA GGC CTG TAC AAT GAA CTG CAG AAA GAT AAG ATG GCG GAG			
GGA GTC CTT CGG GAC ATG TTA CTT GAC GTC TTT CTA TTC TAC CGC CTC			
P Q E G L Y N E L Q K D K M A E>			
1830	1840	1850	1860
*	*	*	*
GCC TAC AGT GAG ATT GGG ATG AAA GGC GAG CGC CGG AGG GGC AAG GGG			
CGG ATG TCA CTC TAA CCC TAC TTT CGG CTC GCG GCC TCC CCG TTC CCC			
A Y S E I G M K G E R R R G K G G>			
1880	1890	1900	1910
*	*	*	*
CAC GAT GGC CTT TAC CAG GGT CTC AGT ACA GCC ACC AAG GAC ACC TAC			
GTG CTA CGG GAA ATG GTC CCA GAG TCA TGT CGG TGG TTC CTG TGG ATG			
H D G L Y Q G L S T A T K D T Y>			

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1930	1940	1950	1960	
GAC GCC CTT CAC ATG CAG GCC CTG CCC CCT CGC AGG AGT AAG AGG AGC CTG CGG GAA GTG TAC GTC CGG GAC GGG GGA GCG TCC TCA TTC TCC TCG D A L H M Q A L P P R R S K R S>				
* * *				
1970	1980	1990	2000	2010
AGG CTC CTG CAC AGT GAC TAC ATG AAC ATG ACT CCC CGC CGC CCC GGG TCC GAG GAC GTG TCA CTG ATG TAC TTG TAC TGA GGG GCG GCG GGG CCC R L L H S D Y M N M T P R R P G>				
* * *				
2020	2030	2040	2050	2060
CCC ACC CGC AAG CAT TAC CAG CCC TAT GCC CCA CCA CGC GAC TTC GCA GGG TGG GCG TTC GTA ATG GTC GGG ATA CGG GGT GGT GCG CTG AAG CGT P T R K H Y Q P Y A P P R D F A>				
* * *				
2070				
* * *				
GCC TAT CGC TCC TGA CGG ATA GCG AGG ACT A Y R S *				

FIG. 8 (contd.)

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FIG. 9

SEQUENCE OF hCTM01 / h / CD28 RECOMBINANT CHIMERIC RECEPTOR

10	20	30	40	
*	*	*	*	
ATG TCT GTC CCC ACC CAA GTC CTC GGA CTC CTG CTG CTG TGG CTT ACA TAC AGA CAG GGG TGG GTT CAG GAG CCT GAG GAC GAC GAC ACC GAA TGT M S V P T Q V L G L L L W L T>				
50	60	70	80	90
*	*	*	*	*
GAT GCC AGA TGC GAT ATC CAG ATG ACT CAG AGT CCA AGT ACT CTC AGT CTA CGG TCT ACG CTA TAG GTC TAC TGA GTC TCA GGT TCA TGA GAG TCA D A R C D I Q M T Q S P S T L S>				
100	110	120	130	140
*	*	*	*	*
GCC AGT GTA GGT GAT AGG GTC ACC ATC ACT TGT AGG AGT AGT AAA AGT CGG TCA CAT CCA CTA TCC CAG TGG TAG TGA ACA TCC TCA TCA TTT TCA A S V G D R V T I T C R S S K S>				
150	160	170	180	190
*	*	*	*	*
CTC CTC CAT AGT AAC GGT GAC ACC TTC CTC TAT TGG TTC CAG CAG AAA GAG GAG GTA TCA TTG CCA CTG TGG AAG GAG ATA ACC AAG GTC GTC TTT L L H S N G D T F L Y W F Q Q K>				
200	210	220	230	240
*	*	*	*	*
CCA GGT AAA GCC CCA AAG CTC CTC ATG TAT AGG ATG AGT AAC CTC GCC GGT CCA TTT CGG GGT TTC GAG GAG TAC ATA TCC TAC TCA TTG GAG CGG P G K A P K L L M Y R M S N L A>				
250	260	270	280	
*	*	*	*	
AGT GGT GTA CCA TCT AGA TTC AGT GGT AGT GGT ACT GAG TTC TCA CCA CAT GGT AGA TCT AAG TCA CCA TCA CCA TCA CCA TGA CTC AAG S G V P S R F S G S G S G T E F>				
290	300	310	320	330
*	*	*	*	*
ACT CTC ACT ATC AGT AGT CTC CAG CCA GAT GAT TTC GCC ACT TAT TAT TGA GAG TGA TAG TCA TCA GAG GTC GGT CTA CTA AAG CGG TGA ATA ATA T L T I S S L Q P D D F A T Y Y>				
340	350	360	370	380
*	*	*	*	*
TGT ATG CAG CAT CTC GAA TAT CCA TTC ACT TTC GGT CAG GGT ACT AAA ACA TAC GTC GTA GAG CTT ATA GGT AAG TGA AAG CCA GTC CCA TGA TTT C M Q H L E Y P F T F G Q G T K>				
390	400	410	420	430
*	*	*	*	*
GTA GAA GTA AAA CGT ACG GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAT CTT CAT TTT GCA TGC CCA CCG CCT CCC AGT CCA CGG CCT CCC AGT V E V K R T G G G G S G G G G S>				

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## FIG. 9 (contd.)

440            450            460            470            480  
      \*            \*            \*            \*            \*

GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA GGT GGC GGA GGG TCA CAG  
 CCA CCG CCT CCC AGT CCA CGG CCT CCC AGT CCA CGG CCT CCC AGT GTC  
 G G G G S G G G S G G G G G S Q>

490            500            510            520  
      \*            \*            \*            \*

ATT CAG CTG GTG CAG TCT GGA GCA GAG GTG AAG AAG CCT GGA TCT TCT  
 TAA GTC GAC CAC GTC AGA CCT CGT CTC CAC TTC TTC GGA CCT AGA AGA  
 I Q L V Q S G A E V K K P G S S>

530            540            550            560            570  
      \*            \*            \*            \*            \*

GTG AAG GTG TCT TGT AAG GCA TCT GGA TAC ACC TTC ACC GAC TAC TAC  
 CAC TTC CAC AGA ACA TTC CGT AGA CCT ATG TGG AAG TGG CTG ATG ATG  
 V K V S C K A S G Y T F T D Y Y>

580            590            600            610            620  
      \*            \*            \*            \*            \*

ATT AAT TGG ATG AGA CAG GCA CCT GGA CAG GGA CTC GAG TGG ATT GGA  
 TAA TTA ACC TAC TCT GTC CGT GGA CCT GTC CCT GAG CTC ACC TAA CCT  
 I N W M R Q A P G Q G L E W I G>

630            640            650            660            670  
      \*            \*            \*            \*            \*

TGG ATT GAC CCT GGA TCT GGA AAT ACA AAG TAC AAT GAG AAG TTC AAG  
 ACC TAA CTG GGA CCT AGA CCT TTA TGT TTC ATG TTA CTC TTC AAG TTC  
 W I D P G S G N T K Y N E K F K>

680            690            700            710            720  
      \*            \*            \*            \*            \*

GGA AGA GCA ACA CTG ACA GTG GAC ACA TCC ACG AAT ACC GCC TAC ATG  
 CCT TCT CGT TGT GAC TGT CAC CTG TGT AGG TGC TTA TGG CGG ATG TAC  
 G R A T L T V D T S T N T A Y M>

730            740            750            760  
      \*            \*            \*            \*

GAG CTG TCT TGT AGA TCT GAG GAC ACA GCA TTC TAC TTC TGT GCA  
 CTC GAC AGA AGA GAC TCT AGA CTC CTG TGT CGT AAG ATG AAG ACA CGT  
 E L S S L R S E D T A F Y F C A>

770            780            790            800            810  
      \*            \*            \*            \*            \*

AGA GAG AAG ACC ACC TAC TAC GCA ATG GAC TAC TGG GGA CAG GGA  
 TCT CTC TTC TGG TGG ATG ATG CGT TAC CTG ATG ACC CCT GTC CCT  
 R E K T T Y Y A M D Y W G Q G>

820            830            840            850            860  
      \*            \*            \*            \*            \*

ACA CTG GTG ACA GTG TCT TGT GCC TCA ACG AAG GGC CCG ACT AGT GAC  
 TGT GAC CAC TGT CAC AGA AGA CGG AGT TGC TTC CCG GGC TGA TCA CTG  
 T L V T V S S A S T K G P T S D>

870            880            890            900            910  
      \*            \*            \*            \*            \*

AAA ACT CAC ACA TGC CCA CGG TGC CCA AAA GGG AAA CAC CTT TGT CCA  
 TTT TGA GTG TGT ACG GGT GGC ACG GGT TTT CCC TTT GTG GAA ACA GGT  
 K T H T C P P C P K G K H L C P>

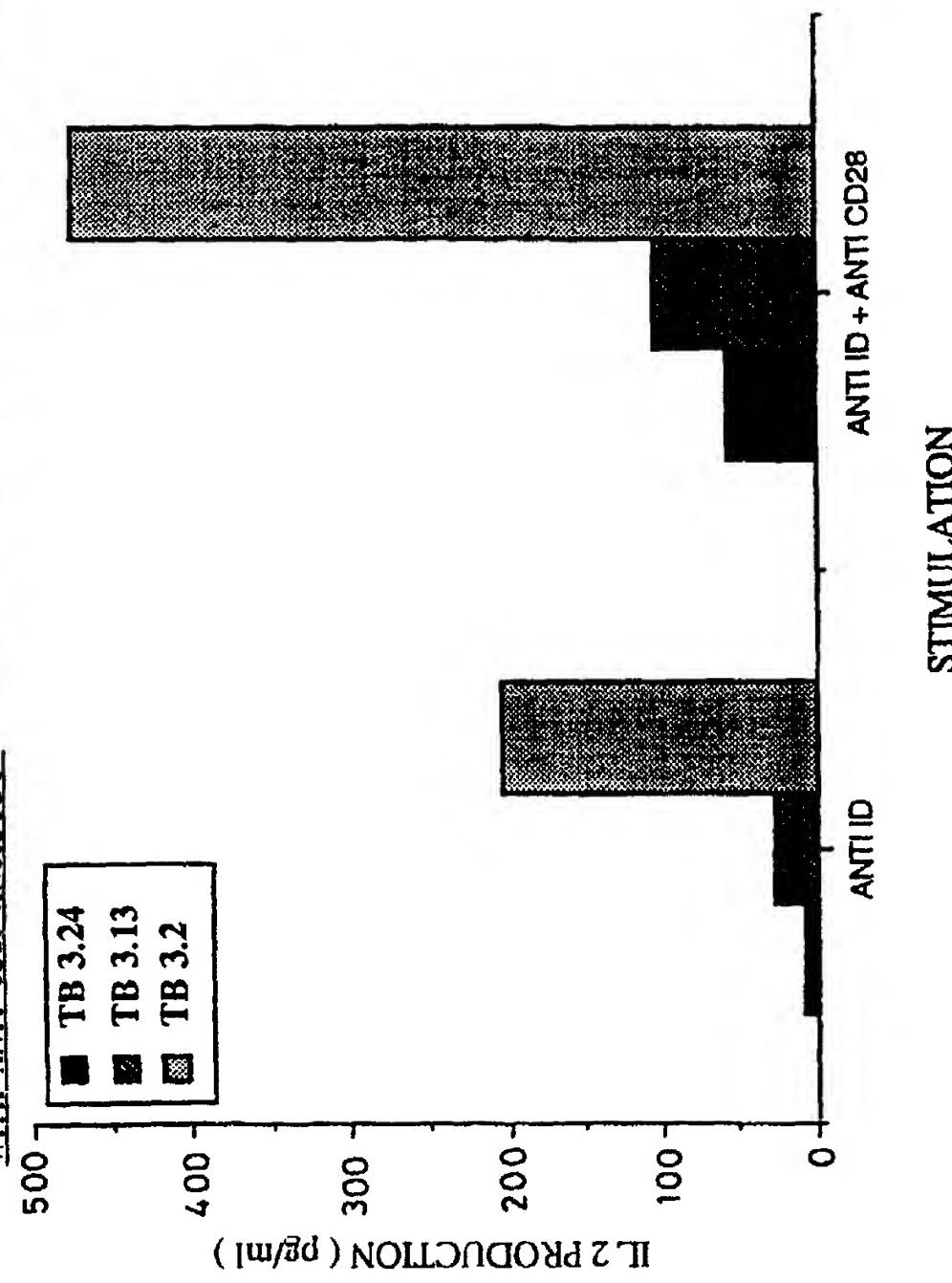
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920	930	940	950	960
*	*	*	*	*
AGT CCC CTA TTT CCC GGA CCT TCT AAG CCC TTT TGG GTG CTG GTG GTG TCA GGG GAT AAA GGG CCT GGA AGA TTC GGG AAA ACC CAC GAC CAC CAC S P L F P G P S K P F W V L V V>				
970	980	990	1000	
*	*	*	*	
GTT GGT GGA GTC CTG GCT TGC TAT AGC TTG CTA GTA ACA GTG GCC TTT CAA CCA CCT CAG GAC CGA ACG ATA TCG AAC GAT CAT TGT CAC CGG AAA V G G V L A C Y S L L V T V A F>				
1010	1020	1030	1040	1050
*	*	*	*	*
ATT ATT TTC TGG GTG AGG AGT AAG AGG AGC AGG CTC CTG CAC AGT GAC TAA TAA AAG ACC CAC TCC TCA TTC TCC TCG TCC GAG GAC GTG TCA CTG I I F W V R S K R S R L L H S D>				
1060	1070	1080	1090	1100
*	*	*	*	*
TAC ATG AAC ATG ACT CCC CGC CGC CCC GGG CCC ACC CGC AAG CAT TAC ATG TAC TTG TAC TGA GGG CGG GCG GGG CCC GGG TGG GCG TTC GTA ATG Y M N M T P R R P G P T R K H Y>				
1110	1120	1130	1140	
*	*	*	*	
CAG CCC TAT GCC CCA CCA CGC GAC TTC GCA GCC TAT CGC TCC TGA GTC GGG ATA CGG GGT GGT GCG CTG AAG CGT CGG ATA GCG AGG ACT Q P Y A P P R D F A A Y R S *				

FIG. 9(contd.)

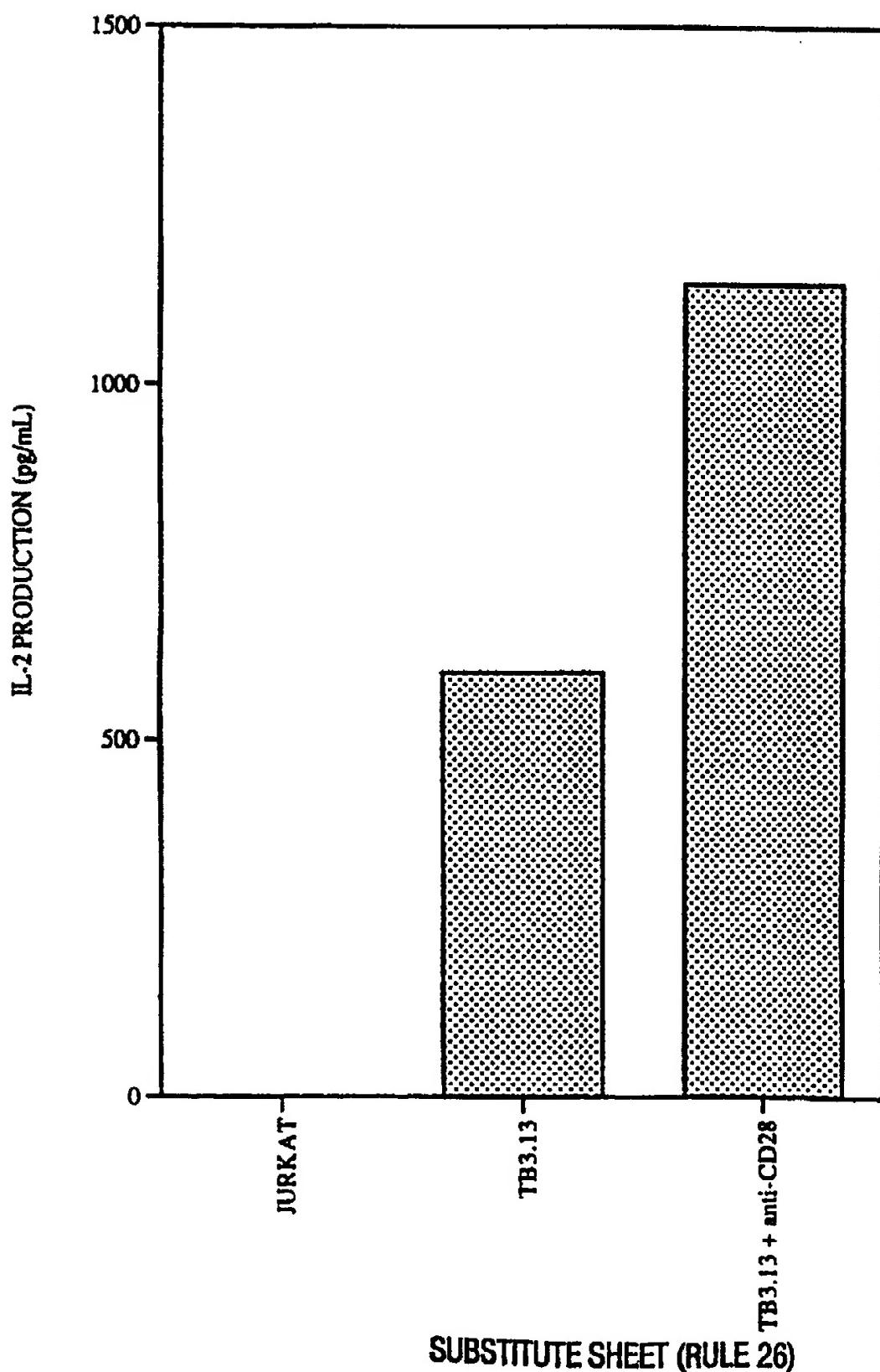
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FIG. 10  
CO-STIMULATION OF CELL LINES EXPRESSING A TCR ZETA CHIMERIC RECEPTOR  
WITH ANTI CD28 ANTIBODY



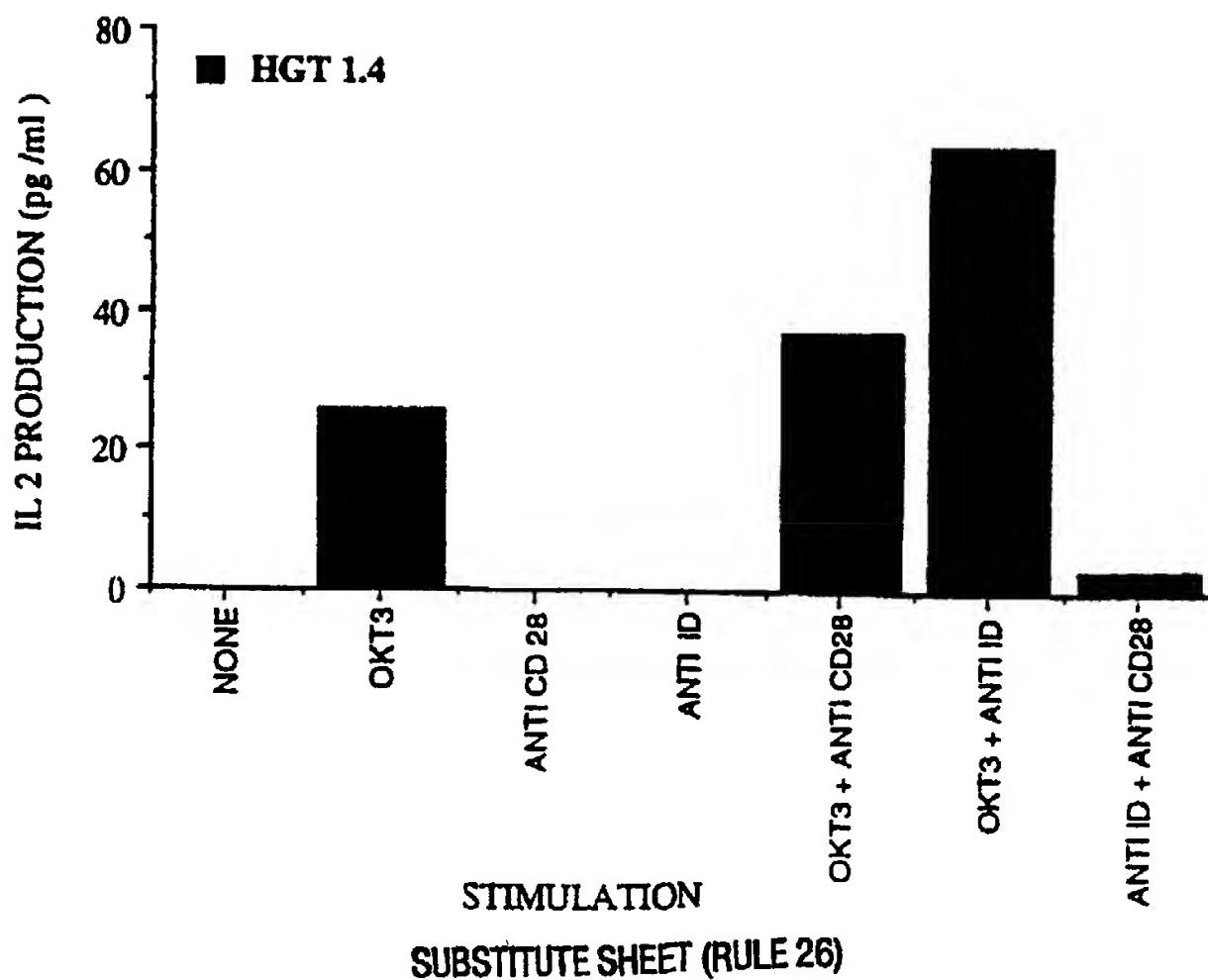
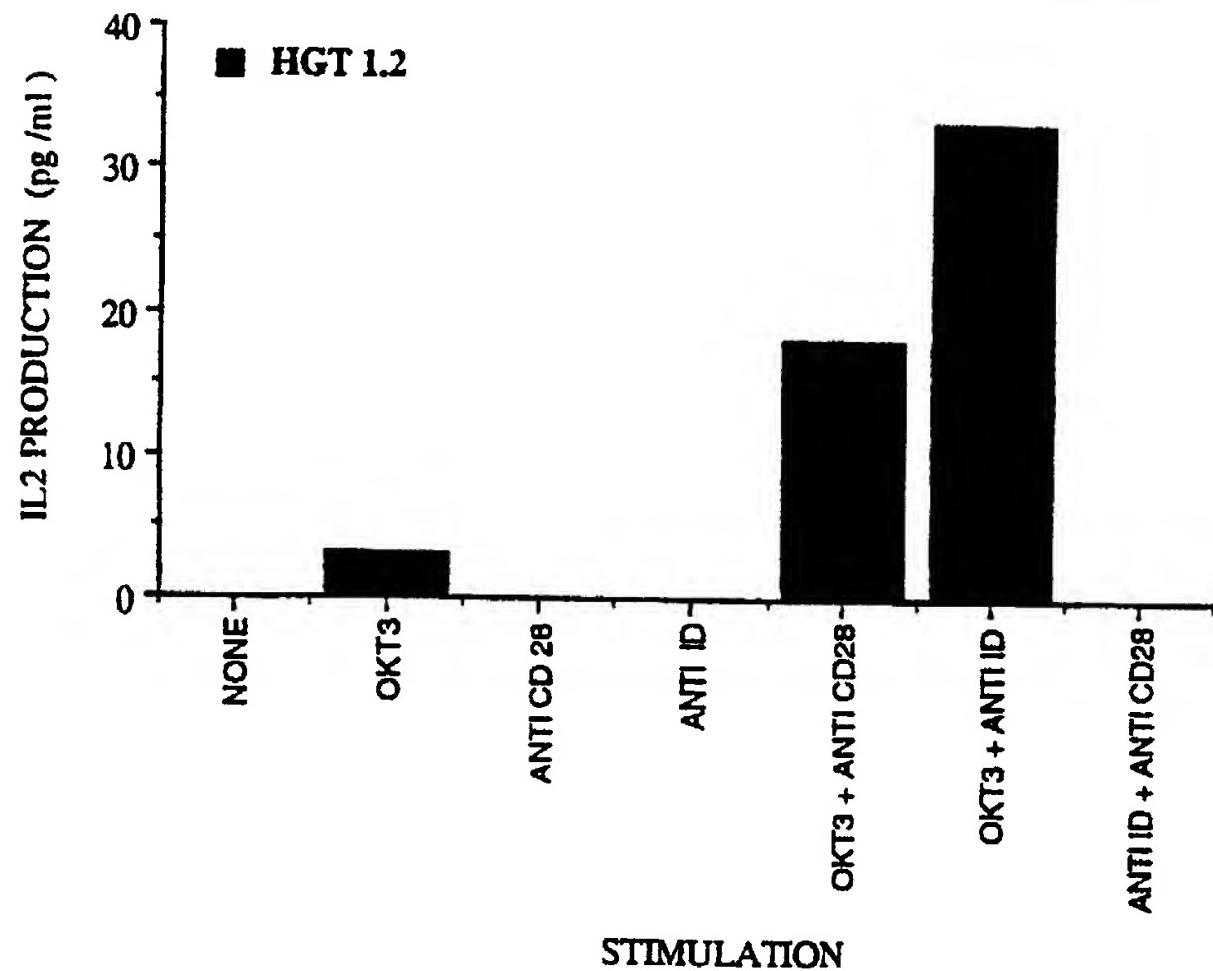
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FIG.11  
STIMULATION WITH ANTIGEN POSITIVE CELLS,MCF-7



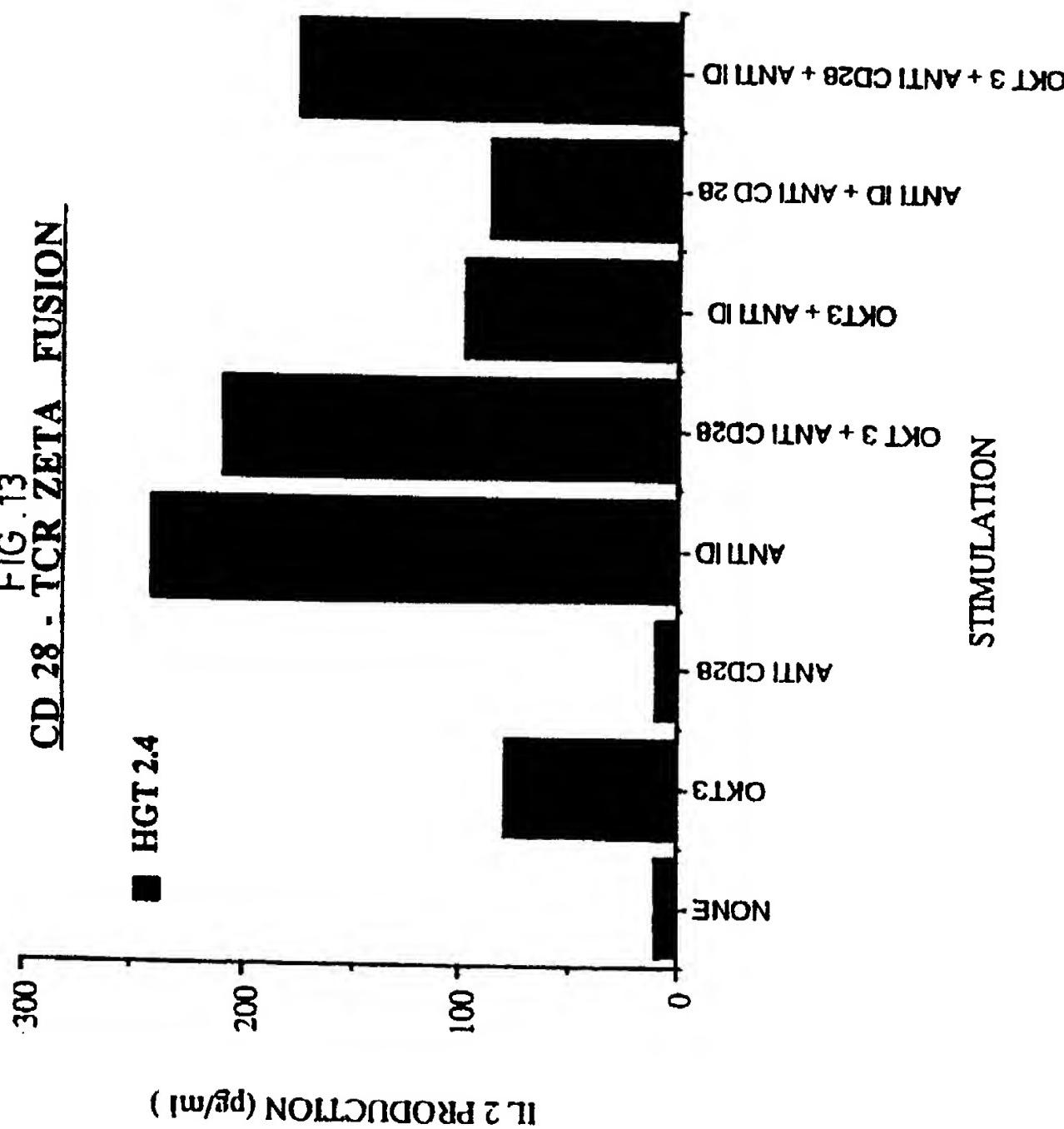
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FIG. 12

IL2 PRODUCTION IN RESPONSE TO VARIOUS STIMULI

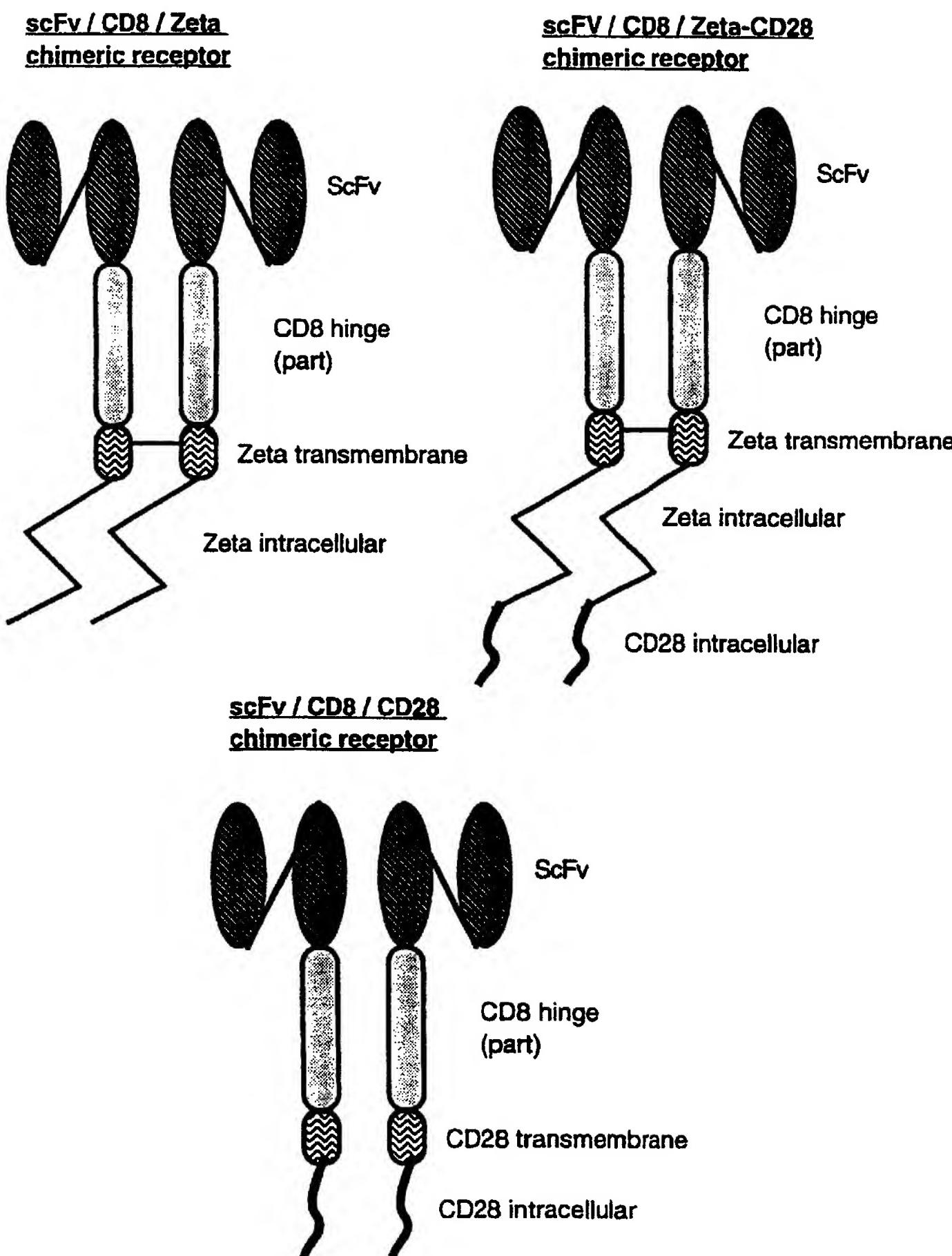
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FIG. 13  
CD 28 - TCR ZETA FUSION



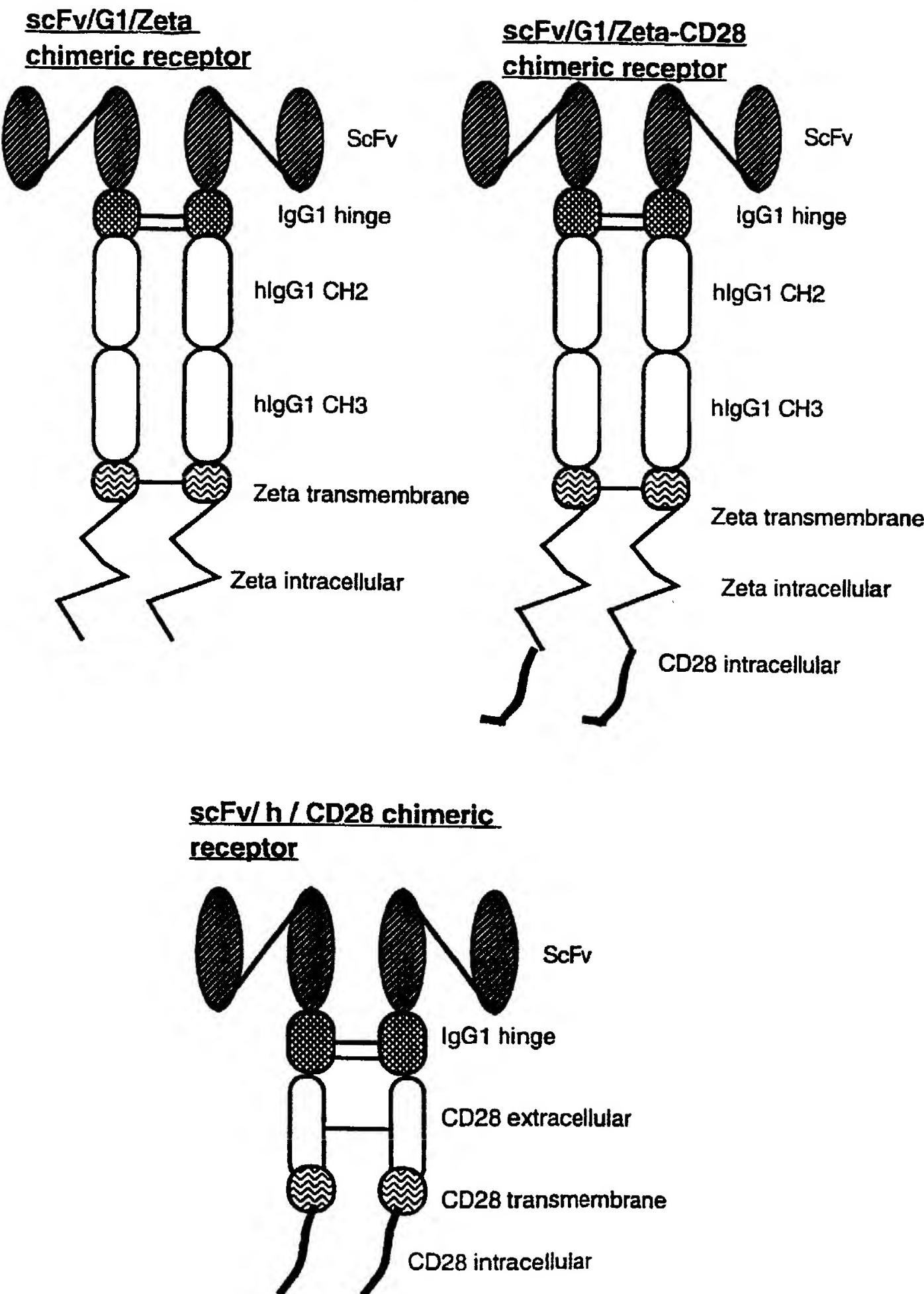
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FIG. 14



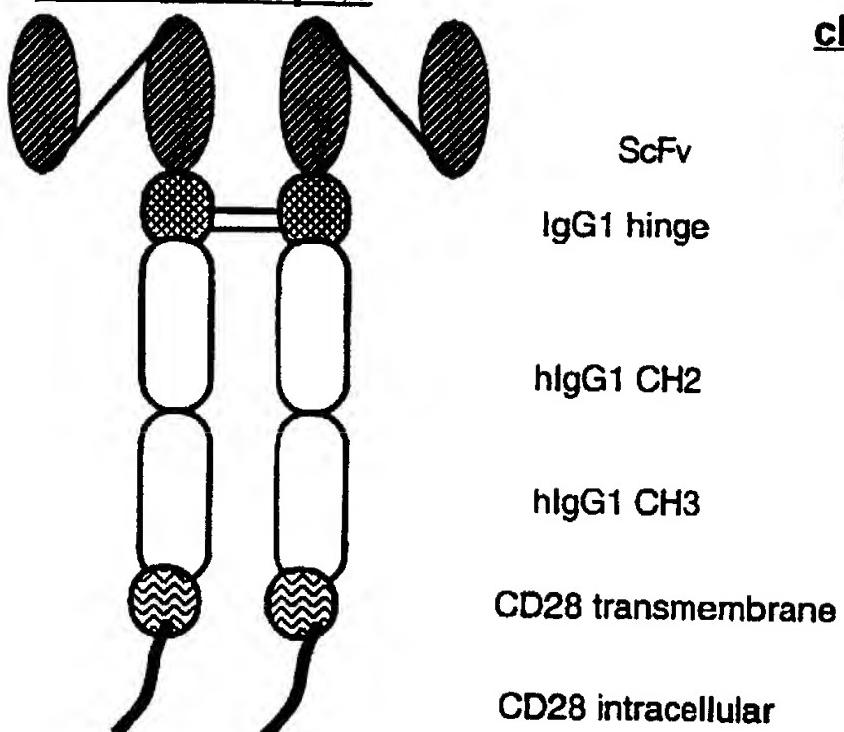
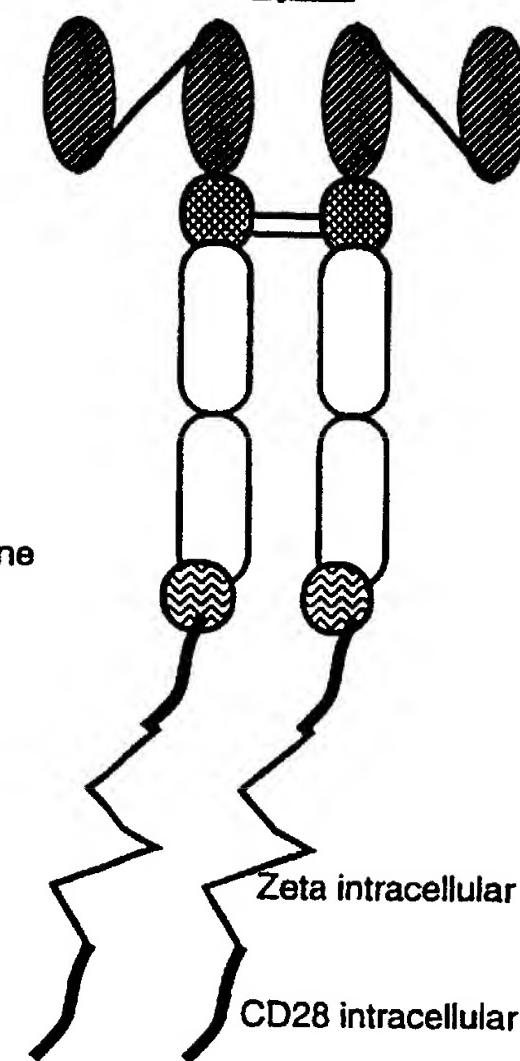
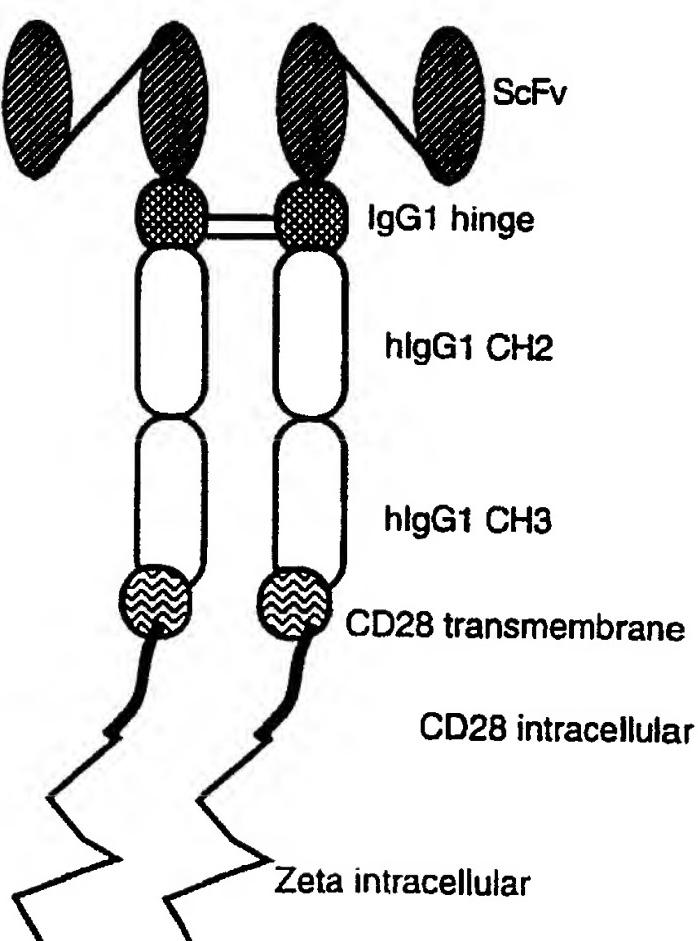
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FIG. 15



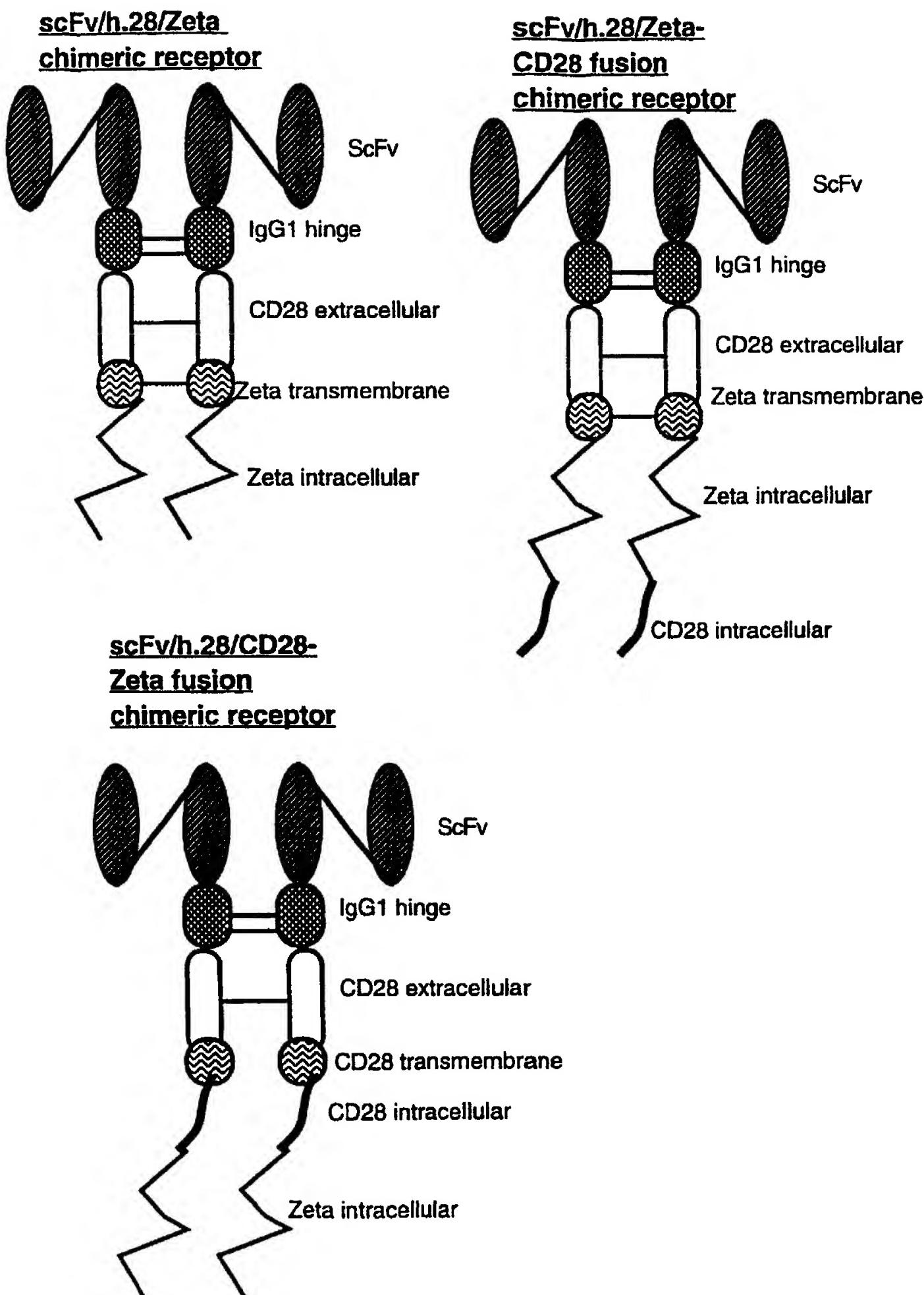
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FIG. 16

scFv/G1/CD28  
chimeric receptorscFv/G1/CD28-  
Zeta-CD28 fusion  
chimeric receptorscFv/G1/CD28-  
Zeta fusion  
chimeric receptor

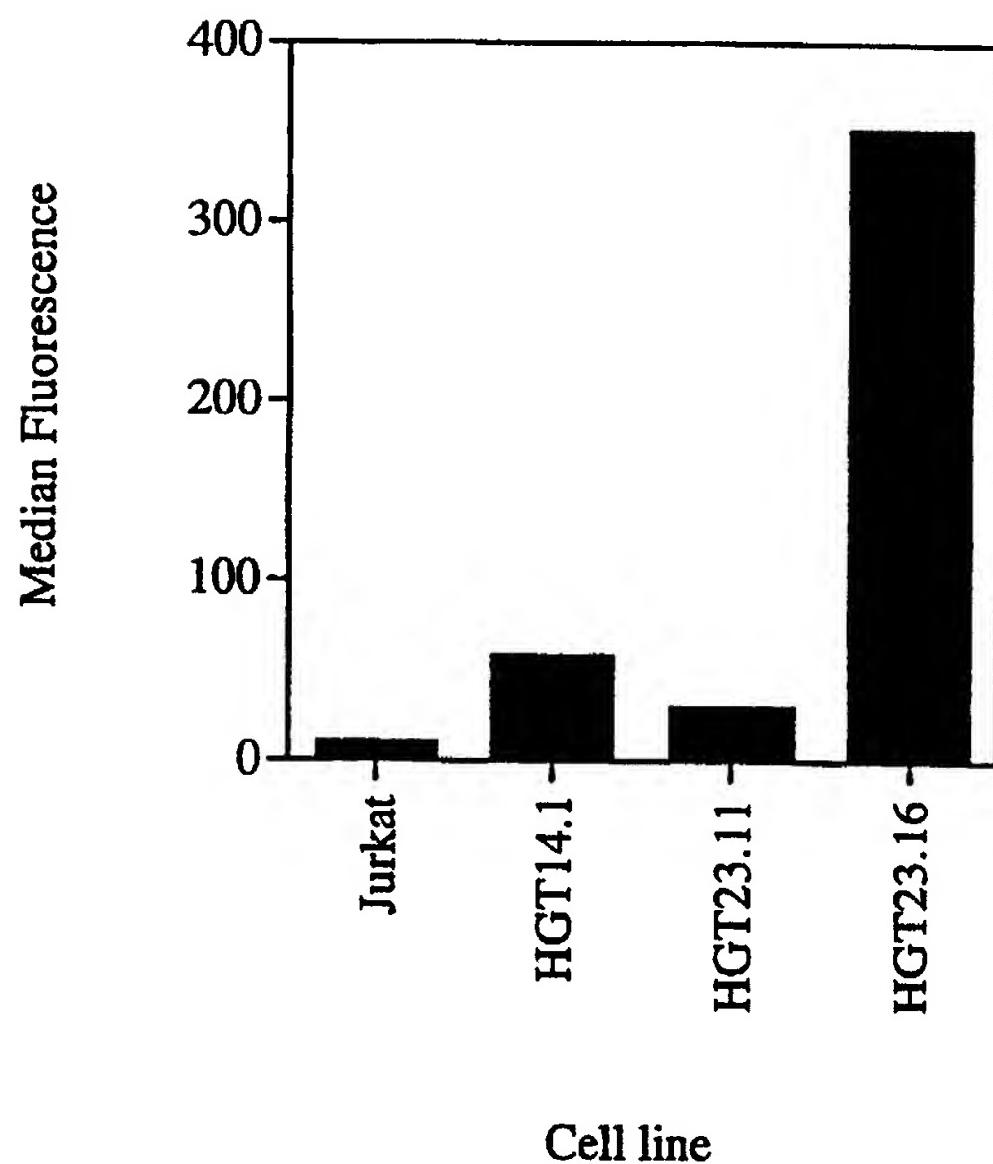
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FIG. 17



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FIG. 18  
Surface expression of CD28-chimeras  
in transfected Jurkat cell lines determined  
by FITC-CD33 staining



**FIG. 19**  
**IL-2 production by Jurkat cell lines expressing p67-CD28 chimeras on infection with RAd160 stimulated with target cells**

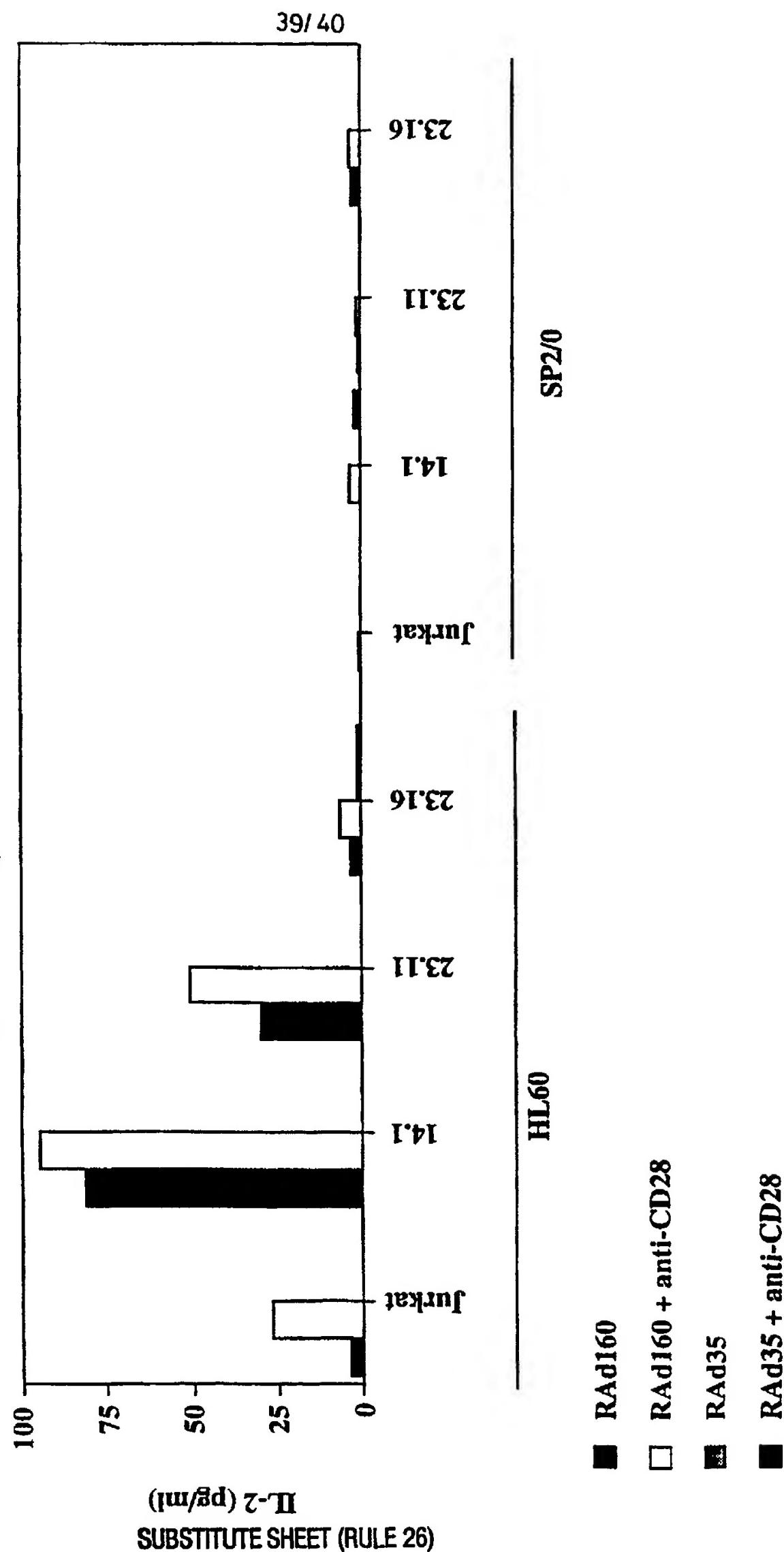


FIG. 20  
**51Cr Release Assay**  
Adenovirus infected CD8<sup>+ve</sup> peripheral  
blood lymphocytes with HL60 target cells

